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LEUCOCHLORIDIUM PROBLEMATICUM N. SP.*

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While examining snails at Fairport, Iowa, in the study of *Lissorchis fairporti* Magath, during the summer of 1917, two snails were found which harbored mature sporocysts of a parasitic trematode of the genus Leucochloridium, and which is to be designated as *Leucochloridium problematicum* n. sp.

The first snail was obtained from Experimental Pond 2 D and was a species of *Succinea retusa* Lea, while the second was a *Planorbis trivolvis* Say, from Experimental Pond 5 D. On further study seven other snails, all of the latter species, were found to be infected with younger sporocysts which could be demonstrated by careful dissection and examination of the livers. In all, records of 209 snails were made, including the following species and numbers of each:

Planorbis trivolvis	120
Succinea retusa	48
Lymnaea obtussa exigua	23
Physa heterostropha	18

This gives an infection of 4.5 per cent. for all; or 2 per cent. for *Succinea retusa* and 7 per cent. for *Planorbis trivolvis*.

In examining the literature for reports of Leucochloridium from America, only two references can be found, and both of them are open to question. The first is that of Call (1898), who remarks under the description of *Succinea obliqua* in Indiana, "the tentacles are rather large and thick, club-shaped, and are often the home of a stage in the development of a planarian."

The second account is given by Ward (1917), who states that Mr. Bryant Walker, in a personal letter, reported finding the larval stage of a Leucochloridium in *Succinea ovalis* in Michigan. It is of course impossible to say whether or not these reports refer to the species or even the genus herein described, but it is possible that since both reports come from nearby states the same worm was found.

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* Contribution from the Laboratory of the United States Bureau of Fisheries, Fairport, Iowa.

Figures 3, 4 and 5 were drawn by Mr. C. W. Shepard, artist in the Department of Anatomy, University of Illinois, College of Medicine, Chicago, Ill.

Leucochloridium is a genus created by Carus in 1835 to contain a species of larval trematode which he named *Leucochloridium paradoxum*. However, Zeller (1874) showed that this larval form developed into the adult trematode that Rudolphi found in the cloaca of *Motacillae lusciniae* in 1803. This early parasitologist called the worm *Fasciola macrostoma*, but in 1809, recognizing its difference from the liver fluke of the sheep, changed the genus name to Distomum, designating the worm as *D. macrostomum*. Monticelli (1888) thought that the description of *D. macrostomum* was sufficient to justify creating a new genus to contain it and called the genus Urogonimus naming *U. macrostomus* as the type. He gave practically no description of the genus except to call attention to the fact that the genital pore was posterior. In 1893 he described *U. ceratus* as a new species. Braun (1896) used in his text both Leucochloridium and Distomum in referring to the species of Carus, but apparently accepted the work of Monticelli, and the genus Urogonimus. Looss (1899) also accepted the genus Urogonimus and described a new species, which he called *U. insignis*. At this time he established a new genus to contain *Urogonimus rossittensis* (Mühling). The two genera were then placed in the subfamily Urogoniminae, with Urogonimus as the type genus.

Stiles and Hassall (1898) stated that they were inclined to adopt *Urogonimus* as a genus in view of the fact that the law of priority did not entitle larval genera to be used as names for adults.

It remained for Poche (1907) to make this final step, and he stated that the genus should be known as Leucochloridium, with *L. macrostomum* (Rud.) as the type, which necessitated the substitution of Leucochloridiinae for Urogoniminae of Looss. This of course does away with the original specific name of Carus for the larval form.

The decision of Poche has been accepted by Lühe (1909) who places the genus in the superfamily Distomata Retizus as Group IV, forms with genital opening posteriorly, and gives a clear cut generic description. He states that the only species found in Germany is *L. macrostomum*, the adult of which has been found in *Rallus aquaticus L.*, *Gallinula chloropus* (L.), and *Ortygometra porzana* (L.). The larval form he reports from *Succinea putris* L. Ward (1917) also accepts this disposition of the genus, and it seems to the author to be the best that can be made, since the genus was clearly defined under the name Leucochloridium and could be so recognized.

Apparently the only larval form that has been reported is that of Carus, who first found it in *Succinea amphibia* in 1833 (reported in 1835) on an island in the Elbe. He states that a friend of his found a similar sporocyst in a snail in Halle in 1825, and it has been reported from Leipzig by Heckert. The description of Carus is of course, not complete, and two of his figures are reproduced in the present paper.

He found a snail whose tentacles seemed to be stained green and white; on close study these were seen to be two bodies, not unlike the larvae of certain insects, which could be retracted into the body of the snail or extended into the tentacles. The mass pulsated and was white with green bands all round it, and brown "warts" at the tip. Near the tip several bands were wider than the others. At the proximal end of the green and white body was a thread-like structure which he later traced into the liver of the snail, where were found numerous small knob-like bodies connected to it by other threads. He tore one of the larger sacs open and found it contained about 300 small worms, each in a little sac of its own. On the inside of the sporocyst were little bodies or cells which he said developed into the worms. These were, according to him, one-sixth of a line long and had well developed organs, which he did not describe in a very complete manner. He further called attention to the fact that the individual sacs of the worms had connections with the two suckers. In discussion of his findings he quotes the following interesting note from Rudolphi (1809): "in tentaculis *Helicis putris L.* Augustus Ahrens Halae Septembri corpuscula reperit, quae omnino huc facere et novum genus sistere videntur."

Following this many observations were made upon this worm, by Leuckart, von Siebold and others, until finally Zeller (1874) showed the relation between the larval form in the snail and the adult worm in the cloaca of singing birds, found many years before by Rudolphi.

What Zeller described in brief Heckert (1889) enlarged upon and gave a very extended account of the histology, morphology and life history of the species. He found the larvae not rarely in the snails near Leipzig. The ramifying tubules penetrated the liver of the host; these tubules were filled with a serous fluid and germ-spheres from which the larvae developed. Parts of the tubules extend up into the tentacles, and these portions are highly colored. The tubules have three muscular layers, a longitudinal, a circular and a diagonal layer. Below this dermo-muscular layer is bright green pigment arranged circularly. The sporocyst has the same structure as the tubules. There is an exterior cuticula, a dermo-muscular tube, and inside this a layer of cells which vary in size with the stage of development. Still inside of this is a membrane with distinct cellular elements, the cells of which differentiate and drop off, eventually forming the larvae. The order of development of the organs of the larvae is as follows: genital cells, suckers, pharynx, enteron, excretory system and nervous system.

There is a double ecdysis, but the cuticula remains with the larva, forming a protective covering, with fluid between it and the worm and the suckers connected to the covering. The Sylviidae are the true hosts and the adult is found in the cloaca; eggs are formed at the end

of eight days. The shells are thick, $\frac{1}{30}$ mm. long and at the hinder end of a ciliated comb, there is a powerful cone which acts as a steering organ. When the eggs were fed to Succinea after eight days they were found in the liver; they bored through the stomach wall with their head cones. Heckert further noted that in the adult the genital pore is terminal or dorsal and not ventral.

Zeller (1874) says the larvae are white, the body flat and broader at the anterior end than posteriorly. The integument is thickly covered with fine hairs or cilia. The anterior end of the body is very peculiarly constructed with its collar-like projection of the entire integument, which overlaps the mouth sucker and sticks up high behind. There are great numbers of unicellular glands in this projection. The suckers are large and very powerful, the mouth sucker being larger than the ventral one. The digestive apparatus consists of a powerful pharynx, a very short esophagus and the intestinal crura which describe a graceful arc as they extend to the posterior part of the body. The excretory system has a short contractile terminal bladder. The rest of the system consists of small tubules which seem to end blindly as fine branches.

The genital organs are quite well developed, and both the male and the female organs lie in the posterior part of the body. The two testes are more or less egg-shaped. One lies immediately posterior to the ventral sucker on the right, the other further back and on the left side of the body. The duct from the first leads out towards the other testis. Both ducts join together and the vas deferens passes posteriorly, lying in the posterior part of the body in a spherical cirrus sac, ending in an extensible cirrus at the posterior end of the body. The cirrus is not easily demonstrated in the larvae, although it is prominent in the adult.

The ovary lies between the two testes, nearer the posterior one. It is round in shape and has a short duct leading from it. This receives first the duct by which the spermatozoa enter from the vagina which opens on the dorsal surface anterior to the excretory opening. After this the duct bends and receives from beneath, at right angles, the ducts from the yolk glands. In the larvae these ducts enter the oviduct in a "rounded-body" which was thought to be the shell gland. The oviduct continues as the true uterus, which at first passes to the left and anteriorly to nearly the pharynx, there crosses to the right above the ventral sucker and descends to the posterior part of the body on the right side where it opens near that of the cirrus sac. The uterus is empty in the larvae, but in the adult the eggs are dark brown and measure 0.025 mm. by 0.014 mm.

The description of the adult *L. macrostonum* does not concern us here, but one might state that the adult structure is accurately fore-

casted by the larvae, and that in the ceca of the singing birds they reach a maximum length of 1.8 mm. by 0.80 mm.

However, the description of *L. insignis* is important for us, and following is given in brief Looss' (1899) description of the species. The worms were found in the cloaca of *Fulica atra*. They are about 3 mm. long and 1.35 mm. broad. The anterior end is thicker and more rounded, the posterior flattened and also somewhat rounded. The suckers are very powerful and large; the mouth sucker has an elevated margin around it. This sucker (0.73 mm.) is as large as, and perhaps larger than, the ventral one. The skin is not ciliated. The mouth leads into a very short prepharynx, and this into a powerful muscular pharynx which is 0.3 mm. in cross section. The esophagus follows immediately. The intestinal crura pass back as far as the genital pore. The excretory pore lies on the dorsal surface 0.27 mm. from the posterior tip. The genital pore is on the dorsal surface 0.17 mm. below the excretory pore. The structure of the genital organs is characteristic for the genus Leucochloridium. The cirrus sac is bulbiform or pear shaped, 0.33 mm. by 0.13 mm. It is a connective tissue mass through which passes the ductus ejaculatorius and from which projects the cirrus; the latter is large in cross section and with thick walls. The pars prostatica is short with but few prostatic glands. The tube decreases and, lying free in the parenchyma is found the thick, muscular-walled seminal vesicle. Into this pass the two ducts from the testes which lie on either side of the body, one behind the other. Between them is the small ovary, lying close to the posterior testes. A receptaculum seminis is lacking. Laurer's canal passes posteriorly and dorsalward to end in the excretory vesicle. The yolk glands lie on the side of the body and stretch from the posterior end of the intestinal crura to the level of the pharynx. The uterus follows the general form of the type species. The egg shell is thick, but not very dark colored, measuring 27μ by 15μ.

Lühe (1909) has defined the genus Leucochloridium as follows: Small distomes, thickened at the rounded end, oval in cross section, with well muscled bodies. Large, very strong suckers. Skin ciliated or smooth. Pharynx strong, esophagus very short. Intestinal ceca very thin and reaching to the posterior end. Excretory pore a little distance from the posterior tip on the dorsal surface. Excretory bladder simple, short. Genital opening at the end. Cirrus sac posterior but enclosing the cirrus and ductus ejaculatorius only. The short prostatic part is also muscular, a spindle shaped seminal vesicle lying free in the parenchyma, passes laterally and obliquely to the posterior part of the body from the testes; the ovary lies between the testes. A receptaculum seminis is lacking. Laurer's canal is present. The yolk glands are numerous, and placed at the sides of the body,

lateral to the intestinal ceca. The uterus has numerous coils, entirely encircling the sucker (ventral). Eggs numerous, small with thick shells. Habitat: the cloaca of birds.

LEUCOCHLORIDIUM PROBLEMATICUM

The description of the sporocyst was made from two mature sporocysts which were essentially alike in all respects. The sporocyst of this species is 1.4 cm. long by 0.33 cm. wide and pointed at both ends. The proximal end is continued in a thread-like tube which a little distance away has in its course a small fusiform swelling. The tube then continues into the liver of the snail where many small knob-like projections appear in the hepatic tissue and are connected by thread-like processes. The wall of the sporocyst is rather thick and tough, being 6μ thick. It is white and translucent. The distal half of the sporocyst is banded with deep golden red bands which show very accurately in figure A (Plate). Some of these are darker than others and the lighter ones tend to be more yellow. No bands appear beyond the distal half. The proximal ¼ is flecked with bronze spots, minute in size, but readily seen. These flecks are also present for a short distance on the tube projection. By comparison with the sporocyst of *L. macrostomum,* it will be seen that this sporocyst is essentially different in marking and color. They are both near the same size.

A cross section of the wall of the sporocyst is seen in figure 14. It is made up of an outer longitudinal layer and an inner circular layer of muscles; the former becomes divided by diagonal fibers which serve to break them up into bundles. The nuclei are irregular and branching. Pigment is present in the cells beneath the outer layer. The sporocyst is capable of pulsation, when mature projects from the snail's tentacle, and each contains about 100 larvae which are very active when freed in water; each has a little sac covering it which is quite transparent and has connections with the two suckers. The sac in the living material was 2.6 mm. by 1.4 mm.

The larvae themselves are about 2.2 mm. long and 0.85 mm. wide. They are quite active and very transparent. The cuticula is beset with fine cilia and some of the organ systems are best studied from living material. This form has two well developed suckers which are readily seen with a low power lens. The oral sucker is the larger of the two, and has its opening near the anterior end of the body. This sucker is 0.24 mm. wide and 0.4 mm. deep. Its posterior end leads into a rather powerful muscular pharynx which is 0.17 mm. by 1.15 mm. From this the lateral intestinal crura arise and each one passes down on either side of the worm to the level of the opening of the excretory canal. These crura are not especially narrow as in *L. macrostomum,* but are in *L. problematicum* 0.55 mm. in diameter. The ventral sucker

is 0.34 mm. in cross section, is circular and situated about in the middle of the antero-posterior axis.

The excretory system is not unlike that of *L. macrostomum,* and consists of a rather simple set of tubules. The flame cells are situated throughout the body and seemed to be collected in a larger pair of tubules, one right and one left. These pass down in the body parenchyma on the ventral side of and median to the intestinal crurae to within 0.10 mm. of the end of the crura. Making a rather sharp turn each tubule, expanding in diameter, passes anteriorly and laterally to the crura to within 0.2 mm. of the anterior tip of the body. Another sharp turn here occurs and with increasing diameter each tubule passes posteriorly between the ascending ramus and its corresponding one of the intestinal crura gradually towards the mid line, where it joins its fellow, after a slight fusiform enlargement, in the mid line 0.09 mm. from the posterior tip on the dorsal side of the body. Almost immediately the excretory pore opens to the exterior. These posterior enlargements of the descending rami are pulsating in character, filling and emptying every few seconds.

The genital organs are quite well developed in these larvae and one has no difficulty in outlining them, as they are certain to occur in the adult. The testes are two in number, one anterior lying to the right of the mid line, the other posterior, 0.13 mm. behind the former and to the left of the mid line. There passes from each and towards the other a small duct, the two joining 0.03 mm. from the posterior testis; from this union there arises a small duct which passes posteriorly, slightly to the right of the mid line, through the cirrus sac and opens as the ductus ejaculatorius to the exterior 0.58 mm. from the posterior tip in common with the uterus. The cirrus sac is fusiform, tapering more sharply anteriorly and is 0.16 mm. by 0.07 mm. No cirrus is developed in this stage of the larvae.

The ovary is spherical in shape, 0.052 mm. in diameter and lies on the left side of the body, at a level between the two testes and nearer the posterior one. There passes from it towards the mid line a short oviduct which almost immediately is joined by Laurer's canal. This canal passes posteriorly and ends in the excretory duct immediately before it opens to the exterior, just as described in the case of *L. insignis* by Looss. The oviduct after a short bend receives the two ducts from the embryonic yolk glands. Following this the oviduct makes a twist upon itself and then passes anteriorly as the ascending uterine branch, first in the mid line, then to the left of the ventral sucker. This branch turns toward the mid line anterior to the sucker and passes posteriorly to the right, then in the mid line and by a more or less straight course to the genital pore on the dorsal surface of the body.

The oviduct receives the yolk gland ducts, which are in reality the shell glands, and makes a coil within an organ called the "round body." This is perhaps a gland which acts in some way upon the shell gland substance, perhaps as a precipitin, and was noted in the larva of *L. macrostomum,* but not in the adult.

Several unicellular glands are found just posterior to the ventral sucker and also along the anterior margin of the oral sucker. Their function is one of speculation, and no data is at hand to make even a profitable explanation for their presence.

TABLE OF MEASUREMENTS

	L. macrostomum Larva	L. macrostomum Adult	L. problematicum Larva	L. insignis Adult	L. cercatum Adult
Length	0.8	1.8	2.2	3.0	4.0
Width	0.45	0.9	0.85	1.35	1.2
Anterior sucker	0.17	0.20	0.39	0.73	0.60
Ventral sucker	0.14	0.20	0.34	0.69	0.72
Width pharynx	0.075	0.16	0.15	0.30	0.22
Testes					
Anterior	0.058	0.20	0.074	0.22	0.26
Posterior	0.060	0.22	0.061	0.20	0.26
Ovary	0.059	0.20	0.052	0.13	0.24
Cirrus sac	0.061 by 0.061	0.15 by 0.16	0.07 by 0.16	0.13 by 0.33	0.13 by 0.34
Round body	0.041		0.043		
Larval sac	1.1 by 0.8		2.6 by 1.3		
Sporocyst	1.7 by 0.25 cm.		1.3 by 0.3 cm.		

All measurements in millimeters.

DISCUSSION

In looking over the description given by Looss of *L. insignis* one is struck with the fact that it is very much like the larval form described in this paper, and the question at once arises as to whether this is in reality the larval form of Looss' species. *Fulica atra* is not found in Fairport, but the corresponding American species is, and it is not impossible that these two trematodes are one and the same. The author, however, does not feel warranted in coming to this conclusion chiefly from a geographical reason, although there is nothing definitely present or absent in the larvae to distinguish it from *L. insignis.* I have, therefore, given it a name symbolic of my conception of the species, and it remains yet to find or experimentally develop adults of *L. problematicum,* which may be compared with *L. insignis.*

In addition to this rather perplexing situation one cannot help but note the similarity between the species of Looss and that of Monticelli. Unfortunately, the description of the latter author is not very complete, and if it is correct and the figure is accurate the form may not even be a member of this genus, because, according to him, the ovary is posterior to both testes, while the genus Leucochloridium is characterized by the location of the ovary between the two testes. However, it seems to me that Monticelli has made an incorrect observation on this point, and the organ labeled "posterior testis" is really the ovary. Granting this, the two forms are then very closely related

and come from the same bird genus. The general size of the worms and their organs are very nearly alike, and the shape of the cirrus sacs are also similar. In the absence of detailed information concerning the species and the fact that Looss, being cognizant of the description of Monticelli, considered his different after a study of his own material, makes it hazardous for me to say that these two worms are the same.

It is not possible to say absolutely, therefore, that the larval form *L. problematicum* is or is not the larval form of *L. ceratum* or of *L. insignis,* or still yet whether it is or is not the larval form of both, they being the same species.

CONCLUSIONS AND SUMMARY

1. A larval trematode, from sporocysts found in *Succinea retusa* and *Planorbis trivolvis,* has been described from Fairport, Iowa.

2. This trematode belongs to the genus Leucochloridium, but is unlike the only larval form of this genus ever described. It has been named *Leucochloridium problematicum.*

3. This larva is remarkably like the adult *L. insignis* (Looss) and *L. ceratum* (Monticelli), and it is possible that they are one and the same species.

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Abbreviations Used in Plates

a t	anterior testis	*o m*	oblique muscle
a s	anterior sucker	*ov*	ovary
a ut	ascending uterus	*ovd*	oviduct
c	cirrus	*p*	pharynx
c m	circular muscle	*p t*	posterior testis
c s	cirrus sac	*r*	rounded body
d ut	descending uterus	*u g*	unicellular glands
e d	excretory duct	*u t*	uterus
e p	excretory pore	*v d*	vas deferens
g p	genital pore	*v dt*	vitillarian ducts
i	intestinal crura	*v s*	ventral sucker
l c	Laurer's canal	*y g*	yolk glands
l m	longitudinal muscle		

The magnification line by the side of each drawing represents a length of 0.2 mm. in all except Figures 13 and 14, in which it is 0.05 mm. long, and Figures 12 and 23, in which it is 0.1 mm. long.

Explanation of Plate IX

Fig. 1.—Toto drawing of *L. problematicum* from the ventral aspect.

Fig. 2.—Drawing of the reproductive organs of *L. problematicum* from the dorsal aspect. × 150.

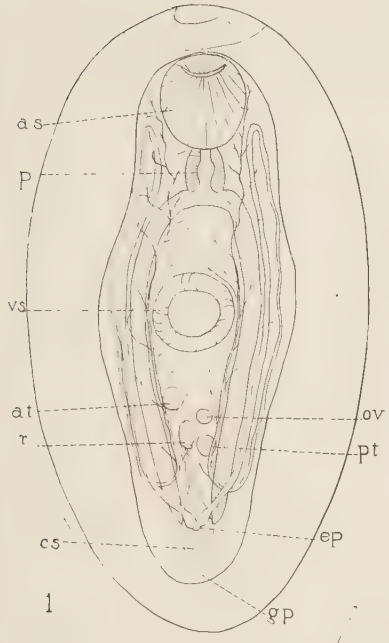
Fig. 3.—Toto drawing of the larva of *L. macrostomum* after Zeller. × 120.

Fig. 4.—Toto drawing of the larva of *L. macrostomum* after Carus.

Fig. 5.—Toto drawing of the adult of *L. macrostomum* after Zeller. × 60.

Fig. 6.—A group of young sporocysts of *L. problematicum* dissected from the liver of *Planorbis trivolvis*. × 8.

Fig. 7.—Longitudinal and transverse sections through *L. problematicum*. Longitudinal sagittal section.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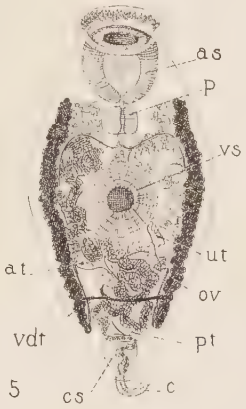
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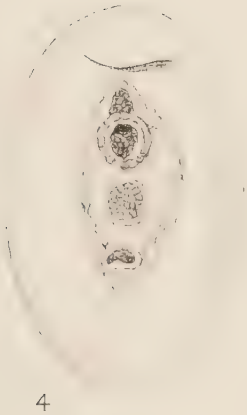
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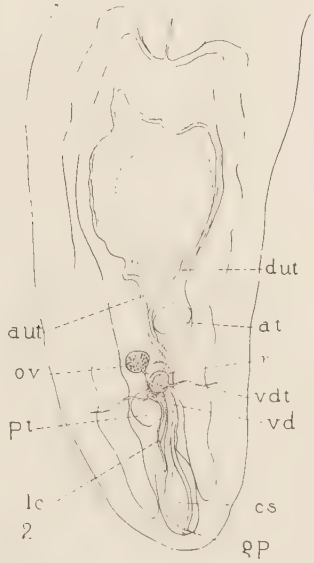
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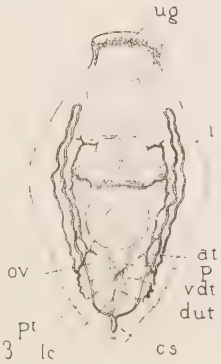
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PLATE IX

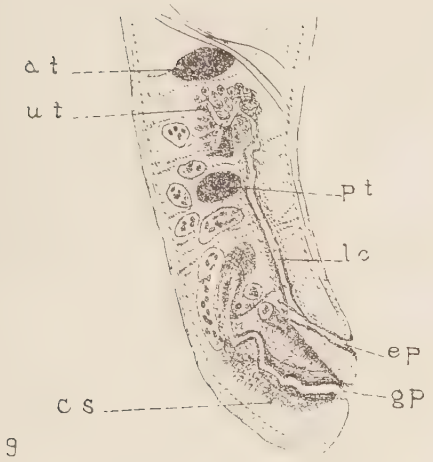
Explanation of Plate X

Fig. 8.—Toto drawing of *L. insignis* after Looss. × 30.

Fig. 9.—Longitudinal sagittal section through the posterior end of the body of *L. insignis*, after Looss.

Fig. 10.—Toto drawing of *L. ceratum* after Monticelli.

Fig. 11.—Posterior region of *L. ceratum*, after Monticelli.



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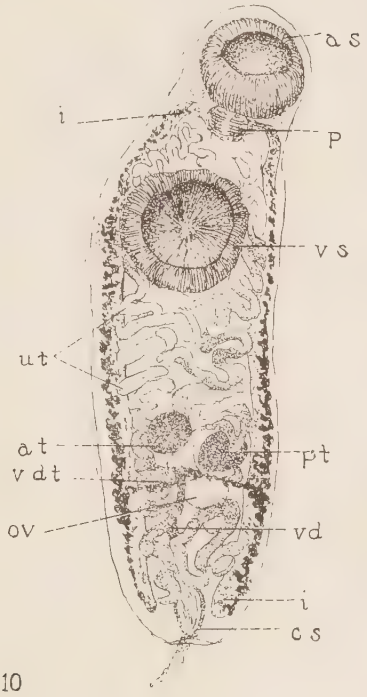

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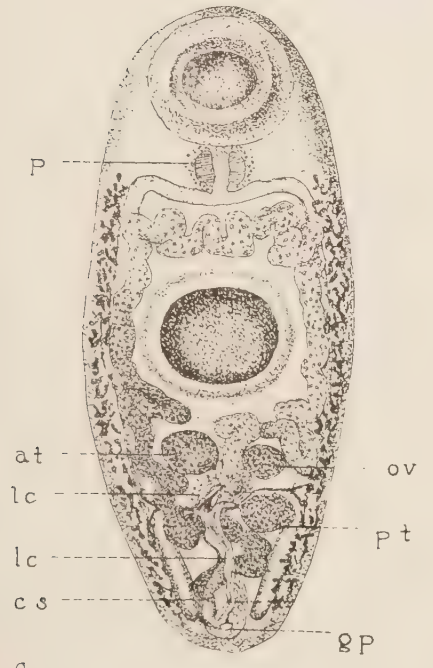

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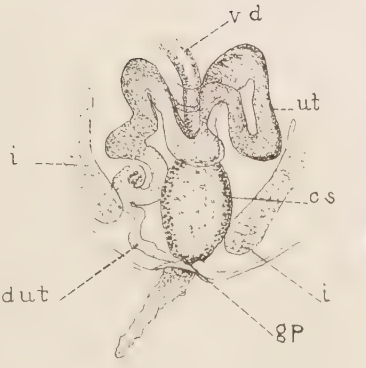

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PLATE X

EXPLANATION OF PLATE XI

Leucochloridium problematicum

Fig. 12.—Longitudinal sagittal section through the anterior margin.

Fig. 13.—Longitudinal sagittal section through the posterior end of the body.

Fig. 14.—Transverse section through the sporocyst wall.

Fig. 15.—Transverse section through the anterior sucker.

Fig. 16.—Transverse section through the anterior sucker, posterior to Figure 15.

Fig. 17.—Transverse section through the ventral sucker.

Fig. 18.—Transverse section through the pharynx.

Fig. 19.—Transverse section through the anterior testis.

Fig. 20.—Transverse section through the ovary.

Fig. 21.—Transverse section through the rounded body.

Fig. 22.—Transverse section through the posterior testis.

Fig. 23.—Transverse section through the cirrus sac.

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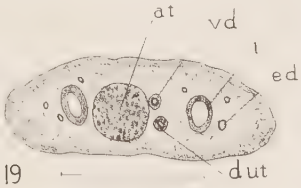

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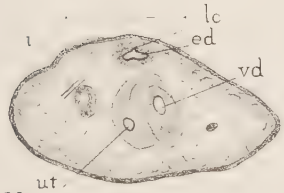

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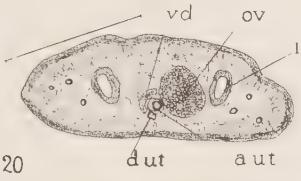

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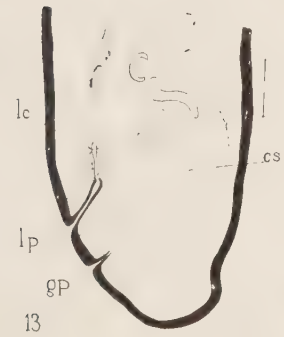

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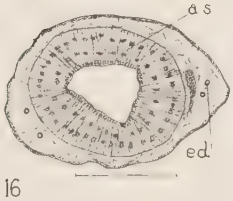

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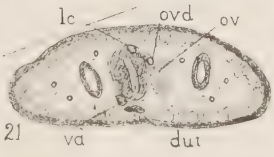

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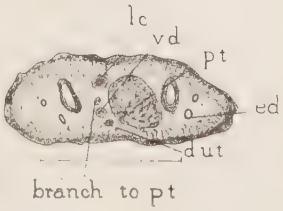

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PLATE XI

THE BIOLOGICAL RELATIONSHIPS OF ASCARIDS

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Zoological Division, Bureau of Animal Industry, United States Department of Agriculture

The experiments described in this paper were undertaken with a view of determining whether, by means of immunological reactions, *Ascaris lumbricoides* which occurs in man can be differentiated from *Ascaris lumbricoides* which occurs in the hog. Morphologically, the forms from the two hosts are indistinguishable so far as present knowledge goes. The name *Ascaris suum* or *Ascaris suilla* which is used by certain writers to designate ascarids which occur in swine is not generally accepted by zoologists for the reason that the classification of animal parasites is based on morphology and not on host relationship. Despite the fact, however, that the specific identity of Ascaris from the hog and from man is commonly accepted on the basis of our present knowledge of the morphology of these forms, much work still remains to be done in order to establish that view beyond any doubt.

The problem which the present writer undertook to solve was whether the apparent morphological identity of *Ascaris lumbricoides* from man and from the hog is correlated with a biochemical identity so far as that can be determined by immunological tests. The solution of this problem necessitated preliminary information as to the possibility of differentiating genera and species of ascarids by immunological methods. The data presented in this paper cover several species of ascarids and throw light on the biological relationships of the forms under consideration.

Flury (1912) made a rather extensive study of the chemistry and toxicology of Ascaris and failed to find any essential differences between *Ascaris lumbricoides* and *Ascaris equorum,* two species that are quite distinct morphologically. Flury employed the methods of analytical chemistry and the usual technic of testing the physiological effects of tissue and organ extracts. The present writer resorted to the more delicate immunological tests by which specific differences may be more readily detected. The differentiation of the fluids of vertebrate species by means of immunological reactions has been studied by many investigators, notably by Nuttall and Uhlenhuth. The former (Nuttall, 1904) writes as follows with reference to the differentiation of the blood of vertebrates by means of cross-precipitin tests:

"The degree and rate of blood reaction appear to offer an index to the degree of blood relationship; in other words, closely related bloods react more powerfully (more precipitum) and more rapidly than do distantly related bloods, provided the latter react at all."

EXPERIMENTS WITH PRECIPITIN TESTS

As is well known the blood serum of an animal immunized to solutions containing proteins acquires the power of precipitating these proteins from solution. Rabbits are commonly used for the purpose of obtaining precipitating serum, and injections are made at intervals of about six days. Four or five injections are usually sufficient to produce a rich precipitin content in the serum.

Following the above technic the present writer immunized a number of rabbits to physiological salt-solution extracts of *Ascaris lumbricoides* from swine. The extracts were made by adding to salt solution pulverized material of entire worms, dried at room temperature shortly after they were removed from the host and washed in physiological salt solution, and extracting for a day or more at room temperature. After filtering the extracts they were preserved with a sufficient quantity of carbolic acid to make a 0.25 per cent. solution. Rabbits were injected intravenously and were bled about a week after the last injection. Small quantities of blood were obtained by severing the marginal ear vein under aseptic precautions. Larger quantities of blood were drawn directly from the heart under ether anesthesia.

Owing to the scarcity of material other than *Ascaris lumbricoides* from swine precipitating serum prepared by immunizing rabbits with extracts of that species only was used. The serum was tested against extracts of several species of ascarids as noted below. The extracts for the tests proper were prepared by adding a definite quantity of dried-worm material to a definite quantity of physiological salt solution and allowing it to extract for a day or longer in a refrigerator. When ready for use the extracts were filtered several times through ordinary filter paper until the filtrates were clear. In each series of tests similar quantities of coarsely pulverized material from each species were extracted in equal quantities of physiological salt solution at the same time and under identical conditions.

Following are the results of the first series of experiments:

The extracts employed in these tests were prepared on the basis of 100 mgm. of dry worm material to 2.5 c.c. of physiological salt solution. The precipitating serum which was used was rather weak.

Five drops of serum were added to tubes containing, respectively, five drops of extract of the following species: *Ascaris lumbricoides* (from swine), *Ascaris equorum, Belascaris marginata,* Toxascaris species (from a wild cat), *Ascaridia maculosa.*

The tube containing an extract of *Ascaris lumbricoides* showed a heavy precipitate in about 20 minutes. The contents of the tube containing an extract of *Ascaris equorum* showed marked clouding two hours after the serum had been added. This was followed by the

settling of a precipitate. The bulk of the precipitate was considerably less than that which settled in the tube containing an extract of *Ascaris lumbricoides*. The contents of the tubes containing extracts of Belascaris and Toxascaris were found to show cloudiness at about the same time, approximately two hours after the serum had been added. The amount of the precipitates formed in these tubes was smaller than that formed in the tube containing the extract of *Ascaris equorum*. The tube containing the extract of *Ascaridia maculosa* remained clear for about four hours during which it was kept under observation. An examination of the contents of the tube the following day showed a very light precipitate, much smaller in amount than those present in the tubes containing extracts of Belascaris and Toxascaris.

These experiments were repeated by using larger quantities of fluids, namely, 25 drops of extracts and 10 drops of serum. After adding the serum the tubes were placed in an incubator at a temperature of 37° C. After 15 minutes' incubation the tube containing the extract of *Ascaris lumbricoides* showed a marked precipitate. The tubes containing extracts of the other species were clear. After being taken out of the incubator the tubes were kept under observation over an hour, but no precipitates developed. The following day precipitates were found in all tubes. Judged by the quantity of precipitate present the tubes ranged in the following descending order: *Ascaris lumbricoides, Ascaris equorum,* Toxascaris, Belascaris and Ascaridia. The tube containing an extract of Ascaridia showed but a slight precipitate.

Additional experiments were carried out with serum diluted in physiological salt solution. Five drops of a 50 per cent. dilution of serum added to five drops of extract of each species referred to above yielded practically the same results as those obtained with pure serum except that precipitates were not noted in the tubes containing extracts of *Ascaris equorum,* Belascaris, Toxascaris and Ascaridia during the period that they were kept under observation (about four hours), while the contents of the tube containing an extract of *Ascaris lumbricoides* became cloudy in about 30 minutes and showed a marked precipitate 30 minutes later. An examination of the tubes the following day showed the presence of precipitates in all tubes except in that containing an extract of Ascaridia. The bulk of precipitate was greatest in the tube containing an extract of *Ascaris lumbricoides*. The tube containing an extract of *Ascaris equorum* was next in order, while that containing an extract of Belascaris showed the smallest quantity of precipitate. Five drops of a 30 per cent. dilution of serum added to five drops of extract of *Ascaris lumbricoides* caused the appearance of cloudiness followed by the formation of a precipitate in less than an hour. Extracts of other ascarids were not tested with this dilution of serum.

Further tests were made primarily with a view of obtaining more data on the differences in the degree and rate of reaction between extracts of *Ascaris lumbricoides* and *Ascaris equorum* by using similar extracts of the two species with equal quantities of serum. The results were uniformly the same, namely, a heavier and more rapidly appearing precipitate in tubes containing extracts of *Ascaris lumbricoides* than in those containing extracts of *Ascaris equorum.*

Each experiment and series of experiments was controlled as follows:

1. Extract of parasite plus a few drops of salt solution.
2. Precipitating serum plus a few drops of salt solution.
3. Normal serum plus a few drops of extract tested.

Unless the controls remained clear the results of the test or of the series of tests were disregarded. As a control on the general specificity of the test for ascarids, precipitating serum was tested against an extract of an unrelated form, namely, *Dictyocaulus filaria,* with negative results.

Inasmuch as the experiments which have just been summarized showed quite conclusively that extracts of the two species of the same genus, namely, *Ascaris equorum* and *Ascaris lumbricoides,* can be easily differentiated by the precipitin test the writer carried out a series of experiments at a later date to determine whether extracts of *Ascaris lumbricoides* from man can be differentiated by the same test from similar extracts of *Ascaris lumbricoides* from the hog. As a control on extracts of these forms an extract of *Ascaris equorum* was tested at the same time. The three extracts were prepared in a similar way, namely, by adding 0.3 gm. of coarsely powdered worm material from each host to 5 c.c. of physiological salt solution and allowing the mixtures to remain in a refrigerator for three days. The precipitating serum used in these tests was stronger than that used in the preceding experiments. The extracts were therefore diluted before being tested, since in the concentrated state differences between the rate of reaction of extracts of *Ascaris lumbricoides* and *Ascaris equorum* were lost.

The extracts were diluted from three to five times with physiological salt solution and 10 drops of extract were tested against 1 and 2 drops of serum, respectively. The tube containing an extract of *Ascaris equorum* did not show any precipitate until at least an hour after the addition of the serum, whereas the tubes containing extracts of *Ascaris lumbricoides* from the two hosts showed precipitates in a few minutes. No differences could be detected in the rate of the appearance of these precipitates, but as a rule the precipitates in the tubes containing extracts of *Ascaris lumbricoides* from swine were somewhat heavier than those in the tubes containing extracts of *Ascaris*

lumbricoides from man. It is doubtful, however, whether that fact has any significance in view of the rather crude manner in which the writer was obliged to prepare his extracts. Since material of *Ascaris lumbricoides* from man was scarce it was necessary to weigh out small quantities which were extracted in correspondingly small quantities of salt solution. It is possible that certain parts of the worm are more soluble in salt solution than others, so that when material from one or two specimens is used the quantity of extract obtained is less than when a similar quantity by weight is extracted from fragments of many specimens. Probably a more accurate method of performing the test would be to use the coelomic fluid of the worms instead of salt-solution extracts. It is expected that as soon as fresh material of *Ascaris lumbricoides* from man is available additional experiments will be undertaken to secure further data on that point.

These experiments were repeated by using more dilute extracts. Thus a dilution of each extract made by adding one part of the extract to nine parts of physiological salt solution and testing 10 drops of the diluted extract against 1 and 2 drops of serum, respectively, yielded the following results: After one hour the contents of the tubes containing extracts of *Ascaris lumbricoides* from the two hosts became cloudy, whereas the tube containing an extract of *Ascaris equorum* was perfectly clear. An examination of these tubes on the following day showed marked precipitates in those containing extracts of *Ascaris lumbricoides* from the two hosts and a slight precipitate in the tube containing an extract of *Ascaris equorum*. A still greater dilution of the extract, namely, 19 parts of salt solution to one part of extract yielded similar results; that is, the tubes containing extracts of *Ascaris lumbricoides* from the two hosts showed precipitates in about three hours, whereas the tube containing an extract of *Ascaris equorum* did not show a precipitate in eighteen hours.

The precipitating serum used in the above-mentioned series of experiments was tested against extracts of Toxascaris species and *Strongylus vulgaris* by adding equal quantities of serum to each extract. Inasmuch as the worm material which was extracted for these experiments was small in bulk the extracts were rather dilute. No precipitate appeared in the tube containing an extract of Strongylus after twenty hours. A very slight precipitate was seen in the tube containing an extract of Toxascaris after a similar period. An extract of *Ascaris lumbricoides* of approximately the same strength as those of the two parasites referred to above, plus an equal quantity of serum, showed a well-marked precipitate about an hour after the serum had been added.

All experiments in this series were controlled as has already been noted elsewhere in this paper.

Summarizing the results of the experiments concerning the relationship of the species of ascarids considered in the foregoing pages, it may be stated that the results of the precipitin tests correspond to the known zoological relationships of these parasites. The differences in the degree and rate of reaction between extracts of two species of the same genus are less than those between extracts of different genera. The slight reactions obtained with extracts of Ascaridia are decidedly significant in view of the fact that that genus is more distantly related to the genus Ascaris than are the genera Belascaris and Toxascaris. The two latter genera are included with Ascaris in the family Ascaridae, whereas the genus Ascaridia belongs to the family Heterakidae. These two families are included in the same superfamily, namely, Ascaroidea. No less significant is the failure to obtain any precipitates with extracts of Dictyocaulus and Strongylus, genera belonging to the superfamily Strongyloidea.

EXPERIMENTS WITH THE ANAPHYLACTIC TEST

The experiments with the precipitin test were supplemented by another series of immunologic tests, namely, by the anaphylactic reaction. The latter is based on the fact that an animal that has received an injection of protein material develops after a certain period a condition of hypersusceptibility to the protein or proteins in question, or, in other words, becomes sensitized to the protein or proteins. A reinjection of material similar to that used in the sensitizing injection calls forth a series of more or less grave symptoms which frequently terminate in death. The anaphylactic reaction is independent of the toxicity of the material injected, since it may be produced by substances that are nontoxic to normal animals.

Without describing in detail the exact response observed by the writer in guinea-pigs sensitized to very small quantities of extracts of various ascarids and reinjected after a period of incubation of two weeks or longer with extracts of the same species as that used for the sensitizing injection and with extracts of related species, the results of several series of experiments involving eighteen guinea-pigs will be summarized briefly. It is important to state in this connection that as a result of the work conducted by various members of this laboratory during the past two years, it was found that guinea-pigs are very tolerant of rather heavy single injections of the body fluids of *Ascaris lumbricoides*. These observations are in harmony with a considerable amount of published data on the effects of the body fluids of ascarids on various animals. The reactions observed by the present writer were considered to be, therefore, anaphylactic reactions and not reactions to any toxic constituents which the fluids of these parasites may contain.

For purpose of convenience the reactions will be referred to as follows:

Mild.—General body tremor, rapid breathing, muscular twitching, scratching of face, etc.

Marked.—In addition to the symptoms above, weakness in legs, frequent defecation and urination, tendency to fall down, etc.

Severe.—In addition to above symptoms, general paralysis.

The results of the experiments follow:

Series A. Six guinea-pigs were sensitized to a salt-solution extract of *Ascaris lumbricoides* from swine by a subcutaneous injection of 0.2 c.c. of an extract made by adding 0.5 gm. of powdered worm material to 15 c.c. of physiological salt solution and allowing the powder to extract for about two hours at room temperature. Fourteen to fifteen days later the animals were reinjected intraperitoneally with 2 c.c. of more concentrated extracts than that used for the sensitizing injection. The results follow:

No. 1. Reinjected with an extract of *Ascaris lumbricoides* from swine. Reaction mild.

No. 2. Reinjected as No. 1. Reaction mild.

No. 3. Reinjected with an extract of *Ascaris lumbricoides* from man. Reaction mild.

No. 4. Reinjected with an extract of Belascaris. No reaction.

No. 5. Reinjected with an extract of *Ascaridia maculosa.* No reaction.

No. 6. Reinjected with an extract of *Ascaris equorum.* No reaction.

Series B. The guinea-pigs used in this series were sensitized by subcutaneous injection of 0.5 c.c. of salt-solution extract of *Ascaris equorum* of about the same concentration as that used in sensitizing the animals of series A. Twelve days after the sensitizing injection the guinea-pigs were reinjected with 2 c.c. of more concentrated extracts as follows:

No. 7. Reinjected with extract of *Ascaris equorum.* Severe reaction.

No. 8. Reinjected with an extract of *Ascaris lumbricoides* (from swine). Marked reaction.

No. 9. Reinjected with an extract of Belascaris. Slight reaction.

Additional experiments were performed as follows:

No. 10. Sensitized to an extract of *Ascaris lumbricoides* (from man). Reinjected twelve days later with an extract of *Ascaris equorum.* Mild reaction.

No. 11. Sensitized to an extract of Belascaris and reinjected twelve days later with an extract of *Ascaris lumbricoides* (from swine). Reaction mild.

No. 12. Sensitized as No. 11 and reinjected thirteen days later with an extract of *Ascaris lumbricoides* (from man). Reaction mild.

No. 13. Sensitized as No. 11. Reinjected with an extract of Toxascaris thirteen days later. No reaction.

No. 14. Sensitized to an extract of *Ascaris lumbricoides* (from man). Reinjected with an extract of *Ascaris lumbricoides* (from swine) twelve days later. Mild reaction.

Series C. The guinea-pigs used in this series were sensitized to an extract of *Ascaris lumbricoides* (from swine). The extract employed for the sensitizing injections was prepared in a similar manner as that described for Series A. From 0.2 to 0.5 c.c. were used in the sensitizing injection which was given subcutaneously. The animals were reinjected eighteen days later with 1 c.c. of concentrated extracts as follows:

No. 15. Reinjected with an extract of *Ascaris equorum*. Severe reaction.
No. 16. Reinjected with an extract of *Ascaris equorum*. Severe reaction.
No. 17. Reinjected with an extract of *Ascaris lumbricoides* from swine. Fatal, death occurring forty minutes after the injection.
No. 18. Reinjected as No. 17. Mild reaction.

The results of experiments with the anaphylactic reaction are not so constant as the results of the precipitin tests, due, no doubt, to the fact that the latter take place in test tubes, whereas the former take place in living animals. Furthermore, the number of animals used is scarcely sufficient to justify any definite conclusions. In a general way, however, more marked reactions were obtained when a guinea-pig was reinjected with an extract of the same species as that used for the sensitizing injection than when it was reinjected with an extract of a related species. It is interesting to observe, also, that the guinea-pigs in series A, which were evidently only slightly sensitized to ascarid extracts, reacted to extracts of *Ascaris lumbricoides* from the two hosts in practically the same way, but that those that were reinjected with extracts of other species gave no reaction.

Attempts were also made to test the series of extracts of ascarids by means of the complement-fixation reaction, but in view of the fact that the extracts employed exhibited a marked tendency to yield non-specific complement fixation and that rabbit serum frequently exhibits anticomplementary properties, that phase of the work was temporarily abandoned.

SUMMARY AND CONCLUSIONS

1. The blood serum of rabbits immunized to salt-solution extract of *Ascaris lumbricoides* (from swine) causes the formation of precipitates when added to salt solution extracts of various ascarids (Ascaris, Belascaris, Toxascaris, Ascaridia). The precipitin reaction as applied to extracts of these parasites is therefore a group reaction.

2. By the use of proper dilutions heavier and more rapidly appearing precipitates are produced when rabbit serum immunized against *Ascaris lumbricoides* is added to salt solution extracts of *Ascaris lumbricoides* than when it is added to similar extracts of other ascarids. Extracts of species of the same genus (*Ascaris lumbricoides* and *Ascaris equorum*) show less difference in that respect than extracts of worms belonging to different genera (Ascaris, Belascaris, Toxascaris, Ascaridia). The results of the precipitin tests correspond, therefore, to the zoological relationships of these parasites.

3. Extracts of *Ascaris lumbricoides* from man do not appear to be distinguishable from extracts of *Ascaris lumbricoides* from swine so far as the results of the precipitin test are concerned. Apparently, the forms from the two hosts are biochemically as well as morphologically indistinguishable.

4. Small quantities of precipitating serum sufficient to cause the formation of precipitates in salt-solution extracts of ascarids failed to produce precipitates in similar extracts of unrelated nematodes (Dictyocaulus, Strongylus).

5. The results obtained by means of the anaphylactic test appear to be in a general way in agreement with the results of the precipitin test, although a considerable degree of variation was noted as regards the reactions of guinea-pigs to injections of similar extracts. Definite conclusions from the experiments on anaphylaxis are not justified in view of the limited number of experiments.

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THE FLAGELLATE CHARACTER AND RECLASSIFICATION OF THE PARASITE PRODUCING "BLACKHEAD" IN TURKEYS—*HISTOMONAS* (GEN. NOV.) *MELEAGRIDIS* (SMITH)*

ERNEST EDWARD TYZZER

The demonstration of the constant presence in the parasite of Blackhead of an extranuclear body which takes part in nuclear division, and the occurrence of a type of nuclear division commonly found in trichomonads (Kofoid and Swezy, 1915) led to the suggestion in an earlier paper (Tyzzer, 1919) of its flagellate character. A subsequent series of observations, to be recorded further on in the present paper, show that this organism may exhibit under certain conditions characteristic flagellate motility.

In the paper above referred to, the author has called attention to the extreme pleomorphism of the protozoon in question, the extent of its morphological variation having no parallel among known parasitic amoebae. The distribution of the various forms of the parasite bears such a relation to the age of the pathological process with which they are associated that they may be interpreted as representing phases of development. Amoebiform organisms with clear blue staining cytoplasm, either with or without inclusions of the nature of ingested material, which occur in great numbers in early lesions and at the periphery of older lesions, were considered to be invasive forms. Organisms having a clear, faintly staining cytoplasm, frequently occurring in such numbers as to greatly distend the tissues and associated with the stage of the disease immediately following invasion were regarded as vegetative forms, although it is quite possible that a large proportion of such organisms present abnormal development and degeneration. A third type of organism found predominating in the older portions of the lesions was interpreted as representing a resistant phase of development. These are relatively small and rounded, present a dense cytoplasm having a marked affinity for eosin and possess a delicate limiting membrane. They are taken up in great number by giant cells.

The multiplication of this organism in the tissues appears to be accounted for by binary fission, at least no evidence of any other type of propagation has been obtained. The process of nuclear division is of the type found in trichomonads. The division centers are derived

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from an extranuclear body, and are connected with each other by a well developed rod-like paradesmose. No multinucleated forms have been found. Parasites with disk-like bodies in the cytoplasm have been interpreted by Smith (1915) as probably representing multiple agamic division. He evidently concludes that this species multiplies by a process which bears a striking similarity to the endogenous spore formation described in amoebae and Balantidium by Walker (1908). Organisms containing these disk-like inclusions are of common occurrence and have been carefully studied in the course of the present investigation. These inclusions vary in size from barely visible particles to bodies exceeding the nucleus in size, and are in some instances so uniform that the parent organisms bear considerable resemblance to the multinucleated cysts of entamoebae. Since the organisms in which they occur retain their original nucleus and extranuclear body unchanged, and since no evidence has been obtained that these disk-like bodies develop into mature organisms, it has appeared more probable that these inclusions represent either the shadows of ingested material or globules of coagulable material.

The dissemination of the parasite from the primary lesions in the ceca to the liver of the turkey is accounted for by Smith through the transportation within phagocytic cells of the problematical small forms discussed above. It is supposed that these cells, after taking up the small forms of the parasite, may in certain instances pass into the veins when they are carried through the portal system to lodge in the liver. It is difficult on this hypothesis to account for the retention of the organisms in the liver, for it would be reasonable to expect that such small parasites on escaping from the phagocytic cells would be occasionally at least swept in the circulation to the lungs and other organs. That the liver serves as an effective filter for the parasite is shown by the limitation of the secondary lesions of the disease to this organ. The demonstration of an active invasive form of the parasite capable of migrating through any of the softer tissues makes the foregoing hypothesis unnecessary. The parasite infiltrates the involved tissue so extensively that it is inconceivable that the blood vessels should escape, and in fact, organisms have not infrequently been found beneath the endothelium of the veins. The size and physical peculiarities of these invasive forms evidently prevent their passage through the capillaries of the liver.

An attempt has been made by Hadley to show that the parasite of Blackhead is identical with "Trichomonas," an intestinal flagellate, which is treated as a species without further distinction. Apparently artefacts and postmortem changes are interpreted as evidences of invasion of the intestinal mucosa by intestinal flagellates. Not only are intestinal trichomonads incorporated into the life cycle of the

parasite, but also organisms of the Blastocystis type. This author has not succeeded in demonstrating intermediate stages connecting any of the intestinal flagellates with the tissue parasite. The confusion of several intermingled species as the developmental forms of a single species has, strange to say, led him also to a conclusion which is not greatly at variance with what is now appparent concerning the nature of the parasite of Blackhead.

Amoeboid movement with slow change of shape has already been recorded (Tyzzer, 1919), but these earlier observations were made upon material kept in a warm chamber at 38 C., rather than at the body temperature of the turkey. Smith (1915) states that no motility has been observed in parasites studied from time to time in the warm chamber except on one occasion when slight changes of form were observed. During the present season, a series of observations made upon fresh material kept at temperatures ranging from 41 to 42 C., the body temperature of the turkey, has brought to light great activity in certain forms of the parasite. The organisms, in hanging drop preparations of scrapings from the lesions mixed with Locke's solution, remain motile for many hours at this temperature. Different types of organisms are distinguishable in fresh material. The small sized dense forms such as are encountered within giant cells are most conspicuous, but these are in general nonmotile. Large and moderate sized forms with a more or less granular cytoplasm frequently show motility, but others appear degenerated and consist of little more than a vesicle containing a granular mass in which a nucleus is at times distinguishable. Least conspicuous are the clear hyaline forms, and these show the greatest activity. The nucleus is usually distinguishable and an occasional organism is found in which four or five lines radiating from the extranuclear body are plainly visible. There is no sharp distinction between ectoplasm and endoplasm, but when granules are present, they are confined to the vicinity of the nucleus, leaving the cytoplasm otherwise clear.

The motility varies in type not only in different organisms, but in the same one at different times during the period of observation, and may be either amoeboid or pulsating in character. Furthermore, the amoeboid movements may be either continuous or interrupted. In the former case the motility may amount in some instances to a slow change of shape, in others to a more or less continuous flowing of the cytoplasm with snail-like progression. In case the amoeboid movement is discontinuous in type, pseudopodia are extended from the main mass of protoplasm, and the latter may stream into the former. There is extreme variation in the rate of motility. The pseudopodia may be sheet-like with a smooth border, but often show one or several rather sharp processes or spurs and are commonly quite irregular. Their

protrusion is usually quite rapid, but not typically eruptive in character. The pseudopodia may be quickly protruded and retracted. An organism watched over a long period of time has been observed to spread out and become sheet-like. Several pseudopodia may be extended almost simultaneously from various points of the surface. Some large organisms may develop a number of short wave-like projections, but subsequently become rounded and remain quiescent. Some maintain a remarkable degree of activity for long periods of time, and amoeboid movement has been noted in organisms from a lesion kept at room temperature for forty-eight hours. Activity in a degree not observed in parasitic amoebae is frequently observed. Sudden displacement or rotation of the main body of organisms frequently results from the movements of extended pseudopodia or adjacent tissue cells may be forcibly separated and shoved to one side. Occasionally an organism exhibits for a considerable period, cytoplasmic movement of a wave-like character, in appearance suggestive of the boiling of a viscid material.

Rhythmic pulsating movements similar to that of trichomonads have been repeatedly observed. This was first noted in a lesion which had been left over night in a partially dissected turkey. Since the possibility of contamination with intestinal flagellates could not be excluded, this observation was disregarded. Organisms showing similar rhythmic movements were subsequently noted in secondary lesions removed with aseptic precautions from several different birds. Stained sections of these lesions reveal no organisms having the morphology of the common intestinal flagellates. Pulsation was in no case immediately apparent in material obtained from freshly killed birds, but developed after a period of from two to four hours in the warm chamber. Several organisms showing this type of motility were in one instance found with the low power from the movement imparted to surrounding cells and debris. They had the morphology of other typical Blackhead parasites present in the material, but were rotating in a jerky manner. No well developed undulatory membrane or flagella were apparent and the rhythmic movement appeared to be internal. On another occasion an organism was observed from which were protruded rather sharp wave-like pseudopodia. These after a time travelled in one direction over the surface of the body, the movements then became characteristic of those of an undulating membrane and the organism began to rotate rather rapidly. Much of the rhythmic movement observed appeared to consist of movement of the interior of the organism, rather than of the surface or of special appendages. Internal granules and nucleus in such instances oscillate with the pulsations without notable change of position of the organism as a whole and without movement of surrounding material. In one case an organ-

ism showing strong, fairly regular rhythmic movement within, continued to send out pseudopodia in various directions.

All attempts to cultivate the organism have thus far failed. The changes taking place after inoculation into the most favorable medium at present available, have been carefully followed. For thirty hours motility continues unimpaired, and the great number of motile forms gives the impression that multiplication is taking place. Binucleate forms are noted and an occasional pair of organisms suggesting binary fission, but there is subsequently no definite increase in number. Actively motile forms occur, but in progressively smaller number up to fifty-four hours in the culture tube at 37 C., but after seventy-two hours all movement has ceased. During this time the amoeboid forms, a large proportion of which show cytoplasmic granules and occasionally rather refractive globules or vacuoles, are replaced by small sized, nonmotile, spherical forms, which possess a clear hyaline cytoplasm without granules or other inclusions and a fairly readily distinguishable nucleus. It is difficult to determine whether the motile forms give rise to the hyaline resting forms or whether they die off, leaving only the latter present. The former possibility appears plausible at least as there are all transitions between the two types. The small nonmotile hyaline forms show at this time no evidence of degeneration or disintegration. No form of the parasite is to be found in culture tubes kept for five days at 37 C., although fairly well preserved organisms are present at this time in tubes kept at room temperature. These, however, show no motility when placed in the warm chamber at 41 or 42 C., and are evidently dead. Notwithstanding the great number of organisms showing amoeboid movement in the case followed in culture medium, the flagellate type of motility was not observed under the conditions furnished. With the appearance of bacteria in the culture, both motile and resting forms of the parasite quickly disappear even though previously present in great numbers. There is no trace of the parasite after twenty-four hours' incubation in salt solution to which a loopful of the cecal contents is added. The prompt disappearance of the organism in liver lesions undergoing putrefaction is also notable.

These observations are in agreement with previous morphological findings and show quite conclusively that the parasite of Blackhead is a flagellate. The rhythmic agitation of internal structures observed in certain instances may be explained only by the presence of a kinetic apparatus wholly enclosed in the cytoplasm. This in all probability consists of the previously described extranuclear body and radiating filaments which may now be interpreted as centroblepharoplast and intraprotoplasmic flagella. It is a notable fact that one of these filaments is coarser than the others (see Figs. 3 to 7, Tyzzer, 1919), but

whether this represents parabasal body or trailing flagellum remains to be determined. Nothing is known concerning the possible existence of free living flagellated forms of this species, although their occurrence is suggested by the development of pulsating movements in organisms observed in the warm chamber. The recognition of such forms might possibly help to solve the problem of its transmission from host to host. It may at present only be conjectured whether such forms exist or whether the organism has become so adapted to parasitic life that it has for the most part lost its ability to live in flagellate form. Wherever, in histological preparations, it has been encountered on mucous surfaces, there is no apparent acquisition of the more characteristic flagellate type of structure. Although pulsating movement characteristic of trichomonads has been observed in material obtained from noncontaminated secondary lesions of several cases, it was not observed in similar material from another case in which amoeboid movement was maintained for fifty-four hours. The exacting conditions necessary to support this organism for even a brief period outside the body of its host as well as its prompt destruction by bacteria indicate that it possesses no marked resistance to conditions encountered outside the body of its host.

Classification.—Smith originally placed the parasite of Blackhead, on account of its amoeba-like characteristics, tentatively in the genus *Amoeba,* and much later (Smith, 1915) retained the same generic name under a different spelling *Ameba.* The view expressed by Hadley that this organism is identical with a previously described coccidium, *Eimeria avium,* is untenable, and was later abandoned by this author. Doflein's suggestion that the organism as a parasitic amoeba should be included in the genus *Entamoeba* now fails to apply with the discovery of flagellate characteristics. Both Jowett's (1911) and Hadley's (1916, 1917) incorporation of the parasite into the genus *Trichomonas* appears to be based upon a confusion of at least two intermingled species for a single species and is unacceptable without more conclusive evidence.

The proof that this organism is not an amoeba makes necessary its reclassification. Its trichomonad affinities are indicated by the type of nuclear division which it presents, by the number of flagella indicated in the five lines radiating from the blepharoplast and by the character of its pulsating movements which appear under certain conditions so that it may thus be included in the family *Tetramitidae* Saville Kent, 1880, as modified by Chalmers and Pekkola, 1918. The assumption of amoeba-like characters with respect to both movement and ingestion of solid particles together with its ability to invade vertebrate tissues appear to justify the creation of a new genus for this species. In case it should prove to be an aberrant form of a type

species already described, the generic name here offered may then be suppressed. The name *Histomonas* is proposed for this genus, which may be defined as follows:

HISTOMONAS gen. nov. Pleomorphic parasitic Tetramitidae with amoeba-like phases of development within tissues of host. The kinetic structures, associated with blepharoplast, intraprotoplasmic during amoeba-like phase. Nuclear division trichomonad in type with well developed paradesmose.

Apart from the pulsating forms in hanging drop preparations of material from lesions, flagellated stages are unknown. No contractile vacuole, no cytostome observed.

Type species: *Histomonas meleagridis* (Smith, 1895) Tyzzer, 1919.

Syn.—*Amoeba meleagridis* Smith 1895.
Eimeria avium Hadley 1909.
Entamoeba meleagridis Doflein 1911.
Trichomonas eberthi Jowett 1911.
Ameba meleagridis Smith 1915.
Trichomonas Hadley 1916.

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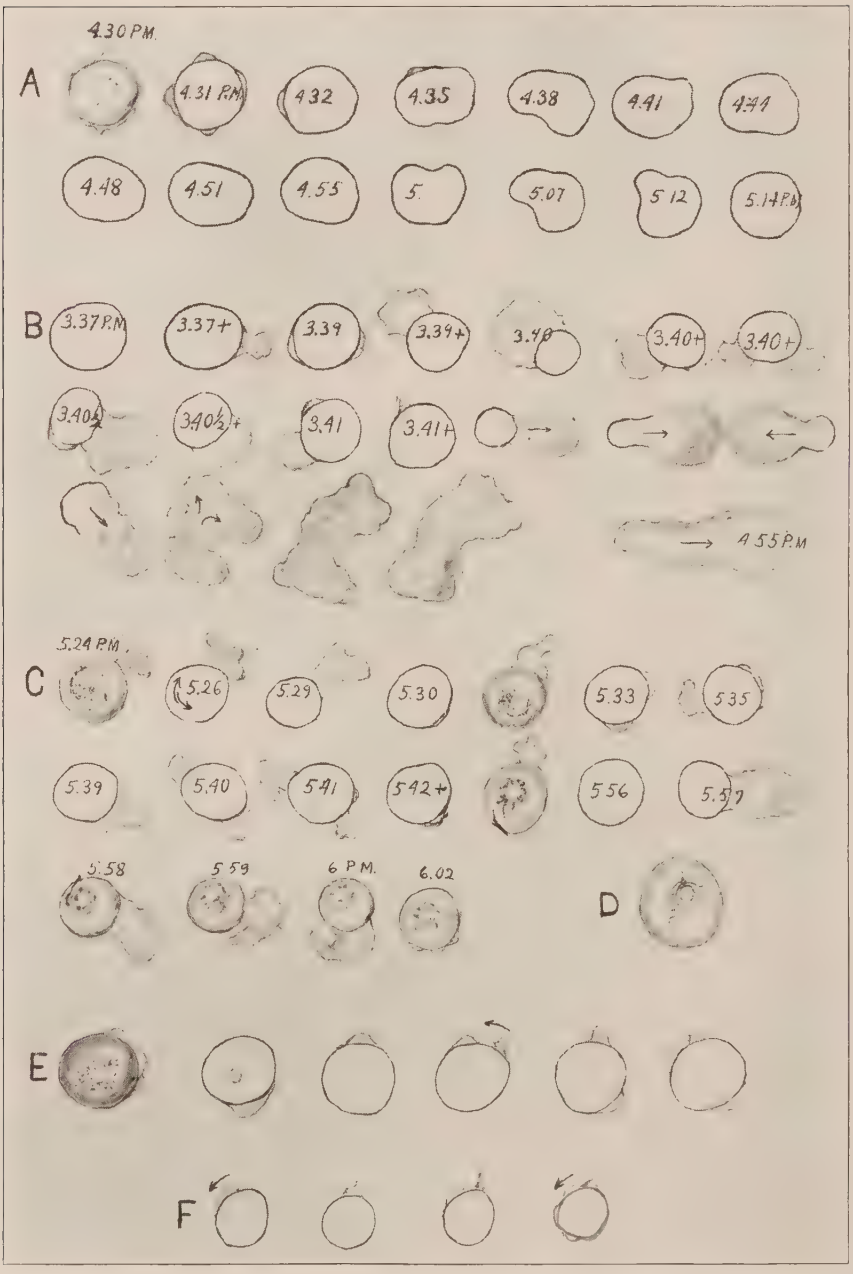
A
4.30 P.M.
4.31 P.M.
4.32
4.35
4.38
4.41
4.44
4.48
4.51
4.55
5.
5.07
5.12
5.14 P.M.
B
3.37 P.M.
3.37+
3.39
3.39+
3.40
3.40+
3.40+
3.40½
3.40½+
3.41
3.41+
4.55 P.M.
C
5.24 P.M.
5.26
5.29
5.30
5.33
5.35
5.39
5.40
5.41
5.42+
5.56
5.57
5.58
5.59
6 P.M.
6.02
D
E
F

PLATE XII

Explanation of Plate XII

Outline sketches to illustrate changes of shape in several different organisms, and in certain instances prominent structural features, observed in hanging drop preparations kept at from 41° to 42° C. in the warm chamber.

A. Different forms assumed by an organism in observations made on Nov. 7, 1919. This organism when first seen showed wave-like pseudopodia. After retraction of the pseudopodia the organism then showed merely slow change of form.

B. Changes of form in an organism observed on Nov. 15, 1919. There was great rapidity of movement with pseudopodia quickly extended and retracted at times from various portions of the surface. The organism eventually spread out in a very thin sheet against the cover glass and moved about rapidly, showing an irregular outline except for a certain period, when it assumed a slug-like form. No pulsation was observed. The nearest approach to this was a quick reversal of movement as indicated in the three sketches following that illustrating the form assumed at 3:41 p. m. This reversal was repeated several times.

C. An organism studied on Nov. 15, 1919. Regular pulsation was noted on first finding the organism about three hours after the preparation was made. The movement consisted of a rotary pulsation of the interior of the organism occurring at regular intervals. Pseudopodia continued to be actively protruded throughout the observation. As the organism rotated upon itself, activity was noted at one point in its surface where there was a slender projecting structure, probably a rudimentary flagellum. This was plainly seen whipping in the surface of the organism at 5:56 p. m., but is not shown in sketch.

D. An organism observed in fresh preparation on Dec. 3, 1919. An extranuclear granule with five radiating lines is visible. In this material no pulsating forms were observed, although the organisms showed active amoeboid motion for fifty-four hours.

E. A parasite followed in observations made on Nov. 7, 1919. First wave-like pseudopodia were observed and in a short time the movement of these became rhythmical and the organism commenced to rotate rapidly.

F. A small organism observed on preceding date of observation. This was first seen sending out rather sharp, wave-like pseudopodia. These traveled in one direction and the organism soon began to pulsate regularly and to rotate actively.

ON THE RESISTANCE OF ASCARIS EGGS

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For several years, in my study on the development of *Ascaris lumbricoides,* I have been testing the resistant power of ascarid eggs against various chemical media in which the eggs were cultured. As far as references go, there are few reports on this subject, notwithstanding it is very important for prevention of ascaris infection.

Method: In the earlier part of my investigation I collected the eggs from the patient's feces dissolved in water, by filtering and then by centrifuging. The collected eggs are treated with a reagent in which the eggs are to be cultured. In the later part, however, I put the fecal mass in the reagent for some time, longer or shorter according to the purpose of experiments, then the eggs were collected from the fecal solution of the reagent by filtering and centrifuging, and lastly were put in a culture dish of the same concentration of reagent as that in which the above treatment is performed.

Culture dishes were kept in the laboratory room in the summer months and in the incubator at 31° C. in the winter.

The vitality of eggs and embryos in each dish was examined at regular intervals of time. The distinction between dead or living eggs was decided partly by microscopical examination and partly by animal feeding experiments. Young or immature eggs were chiefly tested by microscopical observation. The vital power was observed in some cases by transferring the eggs from a reagent into a water culture to test the further development. Even in the same culture there are a great many individual differences in longevity as in other organisms. Thus in the following tables the word "dead" means that the majority of the eggs in the culture indicated are dead, while there are very few eggs alive; and the word "alive" shows a majority of living eggs. Hence it is very difficult to determine exactly the date on which the eggs die or still live in a reagent.

Experiments: During the time from August, 1917, to January, 1918, I carried on a great many experiments, the result of which I published briefly in a Japanese journal of medicine. That is not so important and valuable, for it is all covered by the results obtained in the recent experiments, the results of which are tabulated as follows:

TABLE 1.—AFTER KEEPING THE FECAL MASS IN EACH REAGENT FOR TEN DAYS FROM JANUARY 11 TO 21, 1919, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C.

Reagents	Day							
	13th	18th	28th	31st	38th	43d	52d	
0.5% Nitric acid	E appear	EH normal E alive	do	do	do	do	do	Alive
1% Hydrochloric acid	E alive	do	do	do	do	do	do	Alive
5% Hydrochloric acid	E alive	EH swollen E alive	EH absent present E alive	do	do	do	do	Alive
7% Sulphuric acid	EH absent present E appear	E alive	EH absent or thinned E alive	E alive	do	E shrink		Dead
7% Glacial acetic acid	EH swollen E alive	EH destroyed E alive	EH dest. or absent E alive	E do	do	do	do	Alive
10% Formalin	EH normal E alive	do	E alive	do	do	do	do	Alive
12.5% Formalin	E alive	do	dried up					

E, embryo, EH, albuminous coating.

This table shows that the eggs in 7 per cent. sulphuric acid develop into embryos, but sooner or later they all die. On the twenty-eighth day (February 18) a part of each culture was transferred into the water culture, the further development of which is stated in Table 6.

TABLE 2.—AFTER FOUR HOURS IMMERSION OF FECAL MASS IN EACH REAGENT, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C., ON FERUARY 3

Reagents	Day							
	5th	11th	15th	18th	25th	36th	49th	
0.5% Carbolic acid	F no or rarely 2	EH normal F no	F 2–4 V appear	V	do	do	do	Dead
1% Nitric acid	EH destroyed or absent F many	E appear	EH absent E alive	E alive	do	do	do	Alive
10% Hydrochloric acid	EH normal F many	EH swollen E alive	do	E alive F vacuol	E alive	do	do	Alive
10% Sulphuric acid	EH normal F many	do V appear	E appear	E shrunk	do	E V	do	Dead
10% Glacial acetic acid	EH absent F many	do E alive	E alive F many	do	E alive V appear	E V	do	Dead
15% Formalin	EH normal F many	do V	F V	do	E few	E V	do	Dead
20% Formalin	EH normal F 6–8	.....	F 6–8 V	....	.....	..	..	Dead

F, blastomere; V, vacuole.

The table shows that in 0.5 per cent. carbolic acid or 20 per cent. formalin, ascaris eggs are unable to develop into embryos; in 10 per cent. sulphuric acid or glacial acetic acid, or 15 per cent. formalin, the eggs develop into embryos, but sooner or later die.

TABLE 3.—AFTER FIVE HOURS IMMERSION OF THE FECAL MASS IN EACH REAGENT, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 10

Reagents	Day							
	4th	8th	11th	18th	23d	36th	42d	
0.6% Carbolic acid	EH normal F no	do V	V	do	do	do	do	Dead
1% Corrosive sublimate	EH normal F 4–6	F many	do	E appear	E alive F many	do	do	Alive
1.5% Nitric acid	EH absent or swollen F 2–7	do E appear	E alive	do	do	do	do	Alive
12.5% Sulphuric acid	EH normal F 2–4–6	do E appear	E shrink	do	do	do	do	Dead
12.5% Glacial acetic acid	EH swollen or absent F 2–4–6	do E appear	E alive	do	E alive V appear	E V	do	Dead
15% Hydrochloric acid	EH normal F 1–4	EH ab, or present E appear	E alive F V	do	E shrunken V appear	do	do	Dead
20% Hydrochloric acid	EH normal F 4–7	EH absent or swollen E appear F V	E alive or shrunken	E shrink and V	do	do	do	Dead
25% Formalin	EH normal F no or 2–4	do	F V appear	F V	do	do	do	Dead
Human urine	EH normal F no	do	do	rarely F 2	do	V appear	V	Dead

From this table it is seen that in 0.6 per cent. carbolic acid, or in 25 per cent. formalin, ascaris eggs are unable to develop into embryos; in 12.5 per cent. sulphuric acid or glacial acetic acid, or in 15 per cent. and 20 per cent. hydrochloric acid, the eggs may develop into embryos, but die later; 1.5 per cent. nitric acid is not harmful to the development of the eggs.

TABLE 4.—AFTER FOUR HOURS IMMERSION OF FECAL MASS, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 14

Reagents	Day					
	5th	14th	19th	30th	43d	
0.1% Potassium permanganate	EH normal F no	rarely do F 2–4	E alive	do	do	Alive
0.5% Potassium permanganate	EH normal F no	rarely do F 2–4	E alive	do	do	Alive
1% Iron sulphate	EH normal F many	do E appear	E alive	do	do	Alive
5% Iron sulphate	EH normal F many	do E appear	E alive	do	do	Alive
10% Sulphuric acid	EH normal F many	do E appear	E alive or shrunken	E V	do	Dead
15% Hydrochloric acid	EH normal F many	slightly shrunken	shrunken	V	do	Dead
20% Hydrochloric acid	EH swollen destroy F many	F many V appear	V	do	do	Dead
1% Nitric acid	EH slightly swollen F many	do E appear	E alive	do	do	Alive

The embryos cultured in 0.5 per cent. solution of potassium permanganate emerged from the egg-shell alive. This is a most interesting fact in the study of ascaris development.

TABLE 5.—AFTER TWO DAYS IMMERSION OF FECAL MASS IN THE REAGENT, THE EGGS WERE COLLECTED AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 20

Reagents	Day			
	8th	13th	37th	
0.02% Potassium permanganate	EH normal F no rarely 2-4	F many E appear	E alive	Alive
0.05% Potassium permanganate	EH normal F no rarely 2-4	E alive	do	Alive
10% Iron sulphate	EH normal E appear	F many E alive	E alive	Alive

TABLE 6.—AFTER TWENTY-EIGHT DAYS, THE EGGS WERE TRANSFERRED FROM THE REAGENT (TABLE 1) TO THE WATER CULTURE ON FEBRUARY 18

Date	Reagents				
	0.5% Nitric Acid	5% Hydrochloric Acid	7% Sulphuric Acid	7% Glacial Acetic Acid	10% Formalin
21st day	E alive	E alive	F many E appear	do	F many
34th day	E alive	do	do	do	F many E alive

TABLE 7.—AFTER FIFTEEN DAYS, THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE ON FEBRUARY 18 (See TABLE 2)

Date	Reagents						
	0.5% Carbolic Acid	1% Nitric Acid	10% Hydrochloric Acid	10% Sulphuric Acid	10% Glacial Acetic Acid	15% Formalin	20% Formalin
10th	F no V	E alive	E alive	F shrunk	F many E appear	F many V	V
15th	V	E alive	E alive	F shrunk V	E alive	F V	V
21st	V	E alive	E alive V appear	V	E alive	V	V
34th	V	E alive	V	V	E alive	E no F V	V
	Dead	Alive	Dead	Dead	Alive	Dead	Dead

This table shows that eggs cultured in 10 per cent. hydrochloric acid or sulphuric acid, and in 15 or 20 per cent. formalin died by the fifteenth day of cultivation.

TABLE 8.—AFTER EIGHT DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT, ON FEBRUARY 18 (SEE TABLE 3)

Date	Reagents								
	0.6% Carbolic Acid	1% Corrosive Sublimate	1.5% Nitric Acid	12.5% Sulphuric Acid	12.5% Glacial Acetic Acid	15% Hydrochloric Acid	20% Formalin	25% Formalin	Urine
10th	F no V	F many	F many E alive	F many	E appear alive	F many E appear	E alive or V	V	
15th	V	F many E ailve	E alive	.....	E alive	E alive			
21st	V	E alive	E alive	F shrunk	E alive	E alive	E V	F V	
34th	V	E alive	E alive	shrunk	E alive	E alive	E shrunk	V	F no 2-4
	Dead	Alive	Alive	Dead	Alive	Alive	Dead	Dead	Alive

Eggs in 0.6 per cent. carbolic acid, in 12.5 per cent. sulphuric acid or in 20 per cent. and 25 per cent. formalin were so injured as to be unable to develop further by the eighth day.

TABLE 9.—AFTER FOURTEEN DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT IN THE LABORATORY ROOM, AND PUT IN THE INCUBATOR AT 31° C. ON FEBRUARY 28

Date	Reagents			
	0.1% Potassium Permanganate	0.5% Potassium Permanganate	15% Hydrochloric Acid	20% Hydrochloric Acid
11th day	F many E alive	F no rarely 2-7	shrunken	V
24th day	E alive	E alive	V	V
	Alive	Alive	Dead	Dead

The eggs in 15 or 20 per cent. hydrochloric acid could not develop by the fourteenth day.

TABLE 10.—AFTER TEN DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT CULTURE ON FEBRUARY 28

Date	Reagents			
	0.1% Potassium Permanganate	0.5% Potassium Permanganate	0.5% Carbolic Acid	0.6% Carbolic Acid
23d day	F no rarely 2-4	F no rarely 2-4	F no rarely 2	F no rarely 2
28th day	F many E alive	F many E alive	F no or 2	F no or 2
	Alive	Alive	Alive	Alive

Ascarid eggs retain the power to develop during ten days in 0.5 or 0.6 per cent. carbonic acid.

TABLE 11.—AFTER FOURTEEN DAYS THE EGGS WERE TRANSFERRED INTO THE WATER CULTURE FROM THE REAGENT ON MARCH 11

Date	Reagents							
	7% Formalin	10% Formalin	15% Formalin	20% Formalin	10% Sulphuric Acid	12.5% Glacial Acetic Acid	15% Hydrochloric Acid	20% Hydrochloric Acid
13th	E alive	E alive	E alive	E alive	F	F many	EH absent E F	R alive
18th	do	do	do	do	E alive	E appear F many	E alive	do
	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive

All above experiments are summarized in a table as follows:

Potassium permanganate	0.02% alive	0.05% alive	0.1% alive	0.5% alive	
Corrosive sublimate	1% alive				
Nitric acid	0.5% alive	1% alive	1.5% alive		
Iron sulphate	1% alive	5% alive	10% alive		
Formalin	7% alive until 14th	10% alive	15% dead after 15-18	20% dead after 15-20	25% dead before 8th
Hydrochloric acid	1% alive	5% alive	10% alive	15% dead after 11-14	20% dead after 8, 11 or 14
Glacial acetic acid	7% alive	10% alive, but dead after after 25-30th	12.5% alive until 8th, dead after 28th		
Sulphuric acid	7% alive, dead after 43d	10% dead after 11-15	12.5% dead after 8-11th		
Carbolic acid	0.5% alive 8th, dead after after 11-15	0.6% alive until 10, dead after 8-11th day			
Human urine	Vacuoles appear after 36 or 42, and dead after 70th day				

K. Hotta, a student of our college, also made elaborate experiments on the same subject under my direction during the past year from June, 1918, to May, 1919. The results of his experiments coincide essentially with those of mine, with the exception of a slight difference.

The summarized tables are given as follows:

	Reagents				
	Hydrochloric Acid	Carbolic Acid	Sulphuric Acid	Formalin	Glacial Acetic Acid
Able to develop in	14%	0.3%	9%	12%	8%
Unable to develop in	15%	0.4%	10%	....	9%

Hydrochloric acid	15% alive until 12, 13	17% alive until 12, 11	19% alive until 10	25% alive until 6, 7	28% alive until 5th
Sulphuric acid	10% alive until 12th	12% alive until 11th	15% alive until 11th	20% alive one day	25% dead within day
Formalin	20% alive until 7, 8th	25% alive 7 days	27% alive 6 days		
Carbolic acid	0.4% alive until 30th	0.6% alive 11 days	0.8% alive 10 days		

It is a most important and interesting fact that ascarid eggs are unable to develop and ultimately die in human urine. From some experiments I have assumed that the urine acts more effectively upon eggs at a higher temperature (31° C.) than in the lower (10° C.) for destroying the power of their development.

The influence of the reagent on the ascarid eggs depends upon the permeability of the coverings of the egg. In the above experiments, for instance, formalin and sulphuric acid act to coagulate the albumin-coating of ascarid eggs in consequence of which the penetration of the fluid is prevented. After long action, glacial acetic acid and nitric acid destroy or break down the albuminous membrane of the eggs, but do not penetrate easily through the inner chitinous membrane. For these reasons eggs cultured in those reagents may resist a higher concentration and survive much longer. Hydrochloric acid will also do the same thing.

Carbolic acid, however, may penetrate the egg membrane more easily and more effectively than any other reagents which I have used in the above experiments. Urine contains several kinds of ferments by which the albuminous membrane of the egg may be dissolved. Action of the ferments seems to be accelerated by increasing the temperature as stated above. Moreover, the urine concentration is so much higher than that of the egg-content that the osmotic pressure is sufficiently great to facilitate introducing the urine into the egg-shell.

Besides these experiments on chemicals, I have made some other experiments on the resistance of ascaris eggs to cold.

Exp. 1.—Mature eggs cultured in the incubator from September 28, 1917, to December 11, were put under ground and covered by a thin lawer of soil, and on May 2, 1918, the eggs were given to a guinea-pig which was surely infected.

Exp. 2.—Mature eggs cultured in the incubator from October 27, 1917 to December 22, were put on the ground out-doors, and on May 2, 1918, they were given to a guinea-pig which was also evidently infected.

Exp. 3.—Immature eggs collected on December 8, 1917, were put on the ground from December 13, 1917, to May 2, 1918, then they were transferred to the incubator and kept again in the laboratory room from June to October; next they were put in the incubator during November and December, and in the laboratory room from January to February, 1919, then finally in the incubator from March 1 to 19. On the last date the eggs were given to two guinea-pigs which were killed after a week and showed an infection.

A NEW BI-FLAGELLATED PROTOZOON OF MAN

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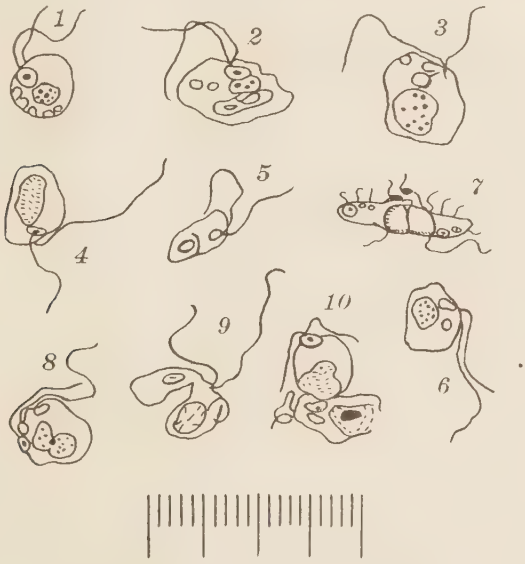
In the study of postmortem cultures and direct smears from various organs of soldiers, we have found what appears to be a hitherto undescribed flagellate protozoon. This organism has been observed in three individuals, occurring respectively in the lung, the sphenoidal sinus, and the heart's blood. Its discovery, in the first place, was more or less accidental. It has been the custom here to supplement bacterial cultures with direct smears from the organs examined in order to study phagocytosis and other cellular phenomena. These smears were fixed in methyl alcohol, stained by Gram's method and counterstained in a weak, watery solution of eosin. Through an oversight a smear from the heart's blood of an influenza patient was left in the eosin solution for several hours; on examining the slide there were found flagellated organisms, and on examining the blood-agar plate culture of the heart's blood, similar structures were observed. Within a few days like protozoa were found in two new autopsies, both in direct smears and in the cultures from the sphenoidal sinus and the lung. Unfortunately the pressure of work during the epidemic of influenza did not permit us to investigate the flagellate closely at that time.

The original smears and slides made from the cultures were preserved after fixation in methyl alcohol; sublimate alcohol fixed slides were lost. Various stains were employed such as Giemsa's, Jenner's, eosin, methylene blue and eosin and iron hematoxylin. It was found that best results were obtained with weak watery solutions of eosin, staining over night. While the Giemsa preparations showed more clearly the internal structures, the flagella were generally so weakly stained that it was difficult to recognize them. It can be seen that our material was not fixed or stained by the best methods, and we are therefore unable to describe in detail the internal structures of the organisms. We realize that our studies are of necessity fragmentary and inconclusive, but we desire to record the findings in order to stimulate search for similar organisms.

MORPHOLOGY

The best preserved organisms were round or pear shaped and measured on an average of 6.5μ in their longest diameter, and 5μ in their greatest width. The extremes of measurements were 6 and 11μ for length, and 3 and 6μ for width. Near the more pointed end could be seen a small kinetonucleus, usually surrounded by a clear area. Sometimes two basal granules whence two flagella took their origin could be recognized near the kinetonucleus; it was a rule for them to have a distinct origin and to be well separated from one another at this



EXPLANATION OF FIGURES

Camera lucida drawings of various forms of the organism described. The small divisions on the scale are each one micron.

Fig. 1 to Fig. 6.—Typical organisms are shown possessing vacuoles, kinetonucleus, trophonucleus with chromatin granules or rods, and flagella.

Fig. 7.—Shows apparently division of trophonucleus. Adherent to the periphery of the protozoon are bacteria-like structures of unknown nature.

Fig. 8.—The trophonucleus is well divided.

Fig. 9.—Shows almost complete cell division.

Fig. 10.—Shows two adult protozoa adherent to one another.

point. These flagella were free, sometimes of equal and other times of unequal length, the shorter averaging 8μ, the longer 14μ. Toward the distal or more rounded end there was a large, trophonucleus, round or oval in shape and averaging 3.5μ in diameter. Coarse chromatin granules or rods could frequently be recognized; in several instances these appeared near the periphery of the nucleus in the form of a dis-

tinct ring. A small, well marked karyosome could often be seen within the nucleus. Within the cytoplasm, were generally several vacuoles; whether these be of contractile nature or food vacuoles we were unable to determine. The outline of the organisms was usually clear and distinct, but adherent to the periphery of several of the specimens were what appeared to be large bacteria.

Several examples were found which seemed to throw some light on reproduction. Thus, we have seen organisms with apparent division of their nuclear structures; others with a deep constriction in their body; and occasionally two fully formed protozoa were adherent to one another. It would seem then that these flagellates divide by binary fission. We also encountered small round bodies, averaging 4μ in diameter, showing the general internal structure, but no indication of flagella; these were looked upon as possible cystic stages.

Living forms were easily observed in cultures on rabbit's blood glycerine agar, on which we were able to cultivate the protozoa to the fourth generation at room temperature. These cultures were plentiful, always in association with various bacteria, chiefly streptococci and pneumococci. The organisms were actively motile, but the use of their flagella was not studied in sufficient detail. In cultures, but never in direct smears from the autopsy material, two other noteworthy structures were observed. One was like a large bacillus, the other like the head of a spermatozoon with a deeply staining granule. Both of these had a well stained yet delicate single flagellum, four to six times as long as their body. These structures might be connected with the genesis of the protozoon, but such a statement necessarily leads into speculation. Sure it is that none of the flagella of bacteria stained with dilute aqueous eosin; many of these bodies resembled the structures adherent to the wall of the protozoa.

DISCUSSION

The organisms described above occurred in every instance in cases of acute influenza. Careful analysis of the history of these patients and of the anatomical changes discovered at the autopsy brings out no additional information. The three patients seemed to have run a disease course exactly similar to that of other influenza patients of that period. As yet we have been unable to find the protozoa in the histologic sections from these cases and we have observed no microscopic tissue changes differing from those of other influenza patients. Klebs has described minute monads and attributed to them some rôle in the pathology of influenza. His observations have, however, not been confirmed. Since we have only found these protozoa in three bodies of 126 influenza necropsies studied in detail, and since the tissue of

these cases presented no unusual alterations, we would look upon the organisms as accidental invaders, possibly from the oral cavity. It is easily seen how such protozoa could make their way from the mouth cavity to the respiratory tract; their presence in the heart's blood can be explained by postmortem invasion from the lungs through the pulmonary veins.

Various flagellated protozoa have been described as occasionally inhabiting the oral cavity or the lungs. Thus, Fantham, Stephens and Theobald state "Prowazek speaks of a variety of *Trichomonas intestinalis* inhabiting the oral cavity. This was distinguished by a posterior process exceeding the length of the body fourfold, and by a somewhat unusual course of the undulating membrane. The food of this form, which was found in the whitish deposit present, especially in the cavities of carious teeth, consisted almost exclusively of micrococci. Schmidt and St. Artault named the Trichomonads found in pathological products (e. g., gangrene, putrid bronchitis, phthisis) of the lungs of man, as *Trichomonas pulmonalis*. Trichomonads have also been found by Wieting in lobular pneumonia in the lungs of pigs."

It is difficult to assign the protozoa which we have described to a definite place because of the insufficient data we possess. The fact that these organisms appear to constantly possess two flagella, places them in the family of Bodonidae, Bütschli, while the possession of a kinetonucleus would place them in the genus Prowazekia, Hartmann and Chagas, 1910. This, however, is only a tentative assignment.

SUMMARY

A small biflagellated protozoon was found in direct smears and in cultures from three postmortems of patients dead from acute influenza. They occurred, respectively, in the heart's blood, sphenoidal sinus and the lung, and apparently produced no tissue changes. The organisms were round or pear shaped, possessed two free flagella and a kinetonucleus. They were easily cultivated on rabbit's blood glycerin agar. We regard these organisms as accidental invaders, possibly belonging to the genus Prowazekia.

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QUELQUES OBSERVATIONS SUR LES PÉDICULIDES

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A l'occasion du typhus exanthématique, qui a sévi au milieu de l'armée et de la population roumaine pendant la terrible guerre, en 1917, j'ai pu faire les observations suivantes sur le pou de corps (*Pediculus vestimenti*).

1. *Les substances recommandées par Prowazek, Versluys, et d'autres, sont inefficaces.*—Aussitôt que le typhus exanthématique a commencé à se répandre parmi la population de la ville de Jassy, j'ai publié un opuscule (1917) destiné à vulgariser l'emploi des diverses substances, que les auteurs recommandaient contre les poux. Déjà les pharmacies mettaient en vente des fioles contenant diverses espèces d'huiles essentielles.

Une fabrique locale de savon, "Carmen Sylva," fabriquait elle aussi un spécifique lancé sous le nom séduisant d'*Exantol*. De leur côté les journaux recommandaient différentes compositions préparées avec des huiles essentielles.

J'ai étudié et expérimenté moi-meme l'action de ces diverses substances sur les poux, afin de constater si, vraiment, l'odeur ou les propriétés chimiques de ces substances avaient pour effet de les éloigner. En 1917, à cause que les poux étaient exanthématiques, je fus obligé de borner mes expériences exclusivement "in vitro." Aujourd'hui (1919), aucun cas de typhus exanthématique n'existant plus à Jassy, j'ai expérimenté la manière du comportement de ces parasites sur mon propre bras. J'ai commencé par faire des expériences avec les substances recommandées par Prowazek: *essences d'eucalyptus, de clous de girofle d'anis.* J'ai pris trois cristallisoirs; dans chacun d'eux j'ai placé 5 poux (*Pediculus vestimenti*) sur un morceau de flanelle. Sur la flanelle d'un des cristallisoirs j'ai fait tomber des gouttes d'essence d'eucalyptus; sur le second des gouttes d'essence de clous de girofle; sur le troisième des gouttes d'essence d'anis. Les poux, non seulement ont continué à vivre de 12 à 24 heures, mais les femelles pendant ce temps ont même pondu leurs lentes. Eysell recommandait en 1915, de saupoudrer la peau avec soufre pilé. J'ai saupoudré mon propre bras et j'y ai placé dessus un pou famélique qui, malgré le soufre, me piqua et me suça. De la même manière se sont comportés d'autres poux, quand j'ai fait des expériences avec de l'essence de térébenthine, recommandée par Marschalkó (1915), avec du baume du Pérou, recommandé par Meltzer (1915), avec de la teinture d'acorus calamus,

recommandée par Versluys (1915) ; ce qui prouve qu'aucune de ces substances n'est d'aucune efficacité contre les piqûres des poux.

2. *Les poux de corps sucent aussi d'autres animaux.*—Galli-Valerio a fait des expériences avec des poux de tête (*Pediculus capitis*), et il a pu démontrer qu'ils sucent aussi d'autres animaux: les cobayes et les souris blanches. Nous avons fait les mêmes expériences avec des poux de corps (*Pediculus vestimenti*). Sur cinq poux que nous avons placés sur un chien, trois ont sucé ; sur quatre placés sur un chat, deux ont sucé ; sur cinq mis sur un lapin, un seul a sucé.

Nous n'avons pas réussi à les faire sucer sur des grenouilles, sur des poules ni sur des pigeons. En tout cas quand on veut faire ces expériences il faut choisir des poux faméliques.

3. *Action des substances grasses.*—Les bergers roumains restent, pendant de longs mois, à garder leurs troupeaux à la montagne, sans jamais changer de linge, et cependant ils n'ont jamais de poux sur leur corps. C'est qu'ils imprègnent leurs chemises et aussi leurs pantalons de laine blanche, qui se trouvent en contact direct avec la peau, de petit lait ou de beurre fondu ; après avoir tordu ces vêtements, pour en exprimer la partie liquide, ils s'en habillent et sont sûrs d' immunité.

J'ai recherché quelle est l'action du beurre sur les poux. Dans ce but j'ai étendu un morceau de flanelle, imprégné de beurre fondu, dans un cristallisoir, et sur cette flanelle j'ai placé un pou femelle (*P. vestimenti*) qui n'avait pas encore pondu les œufs, dont elle était pleine. Dans un autre cristallisoir j'ai disposé un autre morceau de flanelle, non imprégnée de beurre fondu, et sur elle aussi j'ai placé une femelle avant sa ponte. Qu'est-il arrivé? La femelle placée sur ce dernier morceau de flanelle non graissée a pondu et agglutiné chacune de ses lentes d'une manière régulière le long des fils effilochés du tissu ; tandis que celle placée sur le morceau de flanelle imprégnée de beurre a déposé ses œufs sur les fils effilochés sans les y coller. J'ai répété les mêmes expériences avec de l'huile d'olive, de la vaseline, du pétrole, et toujours j'ai constaté que toutes ces substances grasses empêchent le collage des lentes sur les fils du tissu, dont sont faits les vêtements qui en sont imprégnés.

En outre, les substances grasses, en collant les opercules des lentes, tuent les larves, asphyxiées avant l'éclosion ; et les adultes aussi périssent, après quelque temps, les orifices des organes de la respiration restant obstrués par ces mêmes matières.

Parmi toutes les substances grasses, celle qui se trouvait chez nous en plus grande abondance étant le pétrole, je n'ai pas cessé de le recommander à l'occasion de la guerre.

4. *Variétés des poux de corps.*—Souvent il m'est arrivé de recevoir, de la part des médecins d'hôpitaux d'exanthématiques, des échantillons

de poux rencontrés sur le corps des malades, avec prière de les examiner et de leur communiquer s'il est possible d'en distinguer des variétés diverses. J'ai reçu aussi des poux des différents camps de concentration des prisonniers bulgares, hongrois, turcs et allemands.

Ce qui faisait soupçonner à ces jeunes médecins la possibilité de l'existence de plusieurs variétés de poux de corps, c'était l'extrême différence de couleur, de taille, de mouvement que l'on remarquait entre eux, et surtout leurs antennes qui, chez les uns étaient constituées de trois articles et chez d'autres de cinq.

Les poux de corps quand ils sont jeunes ont une couleur jaune-verdâtre; ils peuvent devenir blanchâtres, jusqu'au blond-châtain. Au moment où ils sucent du sang ils deviennent rouges, mais seulement beaucoup plus tard ils deviennent noirs. Cette couleur noire peut avoir deux causes: ou que le sang du tube digestif, après un certain temps, devient noir, et le corps, à cause de sa transparence, paraêt être de la même couleur; ou que le tégument lui-même devient noir.

La taille du pou varie du moment qu'il a quitté l'œuf jusqu'à ce qu'il devient adulte; mais jamais la longueur du mâle ne dépasse les 3 mm. et celle de la femelle les 4 mm.

La vitesse du mouvement chez les poux dépend d'une foule de circonstances. Les poux faméliques cherchent la lumière, tandis que ceux rassasiés, évitent la lumière. Cela explique pourquoi le matin on en trouve sur les vêtements (surtout sur le col) une moins grande quantité que le soir.

Pour ce qui a rapport à la différence des antennes: les poux à trois articles étaient des larves, et ceux à cinq articles étaient des adultes.

De sorte qu'on peut affirmer qu'il existe une seule espèce de pou de corps (*Pediculus vestimenti* Nitzsch-*Pediculus corporis* de Geer) sans aucune variété; espèce qui se distingue seulement de celle du pou de tête (*Pediculus capitis* de Geer).

5. *Les mouches comme agents de transmission des poux.*—J'ai répété l'expérience de Galli-Valerio, en plaçant sous une cloche en verre deux mouches (*Musca domestica*) et un morceau de flanelle sur laquelle étaient déposés plusieurs poux. J'ai saupoudré l'étoffe avec du sucre pour attirer les mouches près des poux. Après 24 heures j'ai trouvé fixé au thorax de l'une d'elles un pou. Elle voletait de-ci de-la sans que le pou tombât. J'ai enlevé les ailes de la mouche et puis je l'ai laissée se promener sur mon bras gauche mis à nu. Le pou, après une quinzaine de minutes s'est détaché du thorax de la mouche en tombant sur la peau de mon bras.

Cette expérience nous paraît suffisante pour nous faire admettre que les mouches peuvent très bien servir de véhicule aux poux.

6 *Le typhus exanthématique ne peut se propager par la poussière.* — Le Dr. Imbert qui jadis fut membre de la Mission francaise d'épidémiologie en Serbie, et qui en 1917 était détaché chez nous, a publié dans les journaux locaux un article dans lequel il exprimait l'opinion que le typhus exanthématique peut se propager par la poussière provenant de la décomposition des poux exanthématiques. Les poux morts—disait-il—et les débris mêmes des poux morts qui se trouvent sur le sol, et qui contiennent encore le virus contagieux, sont émiettés. desséches, pulvérises et puis soulevés en l'air, soit par le balayage, soit par les traînes des robes, soit simplement par la démarche, après quoi ils pénètrent par les narines et par la bouche et sont inspirés.

Eh bien! parmis les nombreux hôpitaux d'exanthématiques installés à Jassy, il y en avait quelques-uns qui se trouvaient précisément au centre de la ville. Un grand nombre de fenêtres de ces hôpitaux s'ouvrent directement sur les rues. Incessamment, surtout au printemps, par une insouciance déplorable, on balayait les salles, les fenêtres extérieures étant grandes-ouvertes, et on secouait dehors la literie des malades; de sorte que la poussière tombait directement sur le trottoir de la rue, partant sur les passants.

Et cependant il n'y eut d'atteints de la terrible contagion que ceux qui, se trouvant en contact direct ou indirect avec les malades, emportaient sur leurs personnes des poux vivants.

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ON A NEW SPECIES OF RHABDITOID WORMS FOUND IN THE HUMAN INTESTINES

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Most species of Rhabditis and its allies are found free living in the earth and decayed vegetable matter. Several examples of parasitic Rhabditis, however, have been reported, e. g., *Rhabditis pellio* (Scheiber) in the urine (Scheiber 1880, Baginsky 1887), *Rhabditis* niellyi (Blanchard) in the skin (Nielly 1882), *Rhabditis* sp. in the stomach (Frese 1907) and others. Such were also found parasitic in certain other species of mammals. They may be true parasites, but most of them seem rather to be facultative parasites.

In 1914, when I was engaged in the microscopical examination of the feces of the pupils of a primary school in Ibaraki Prefecture, Japan, for the eggs of parasites, I found several times in the fresh feces worms belonging to a certain species of Rhabditis. It seemed to be new to science and to it I gave the name *Rhabditis hominis* n. sp.; it is this with which I am going to deal in the present communication.

Rhabditis hominis is viviparous and found numerously in the freshly passed feces (strict precautions were taken to exclude free living nematodes). All the stages of its development occurred in the same specimen of feces, i. e., the adults and young of both males and females and the newly hatched larvae.

A full grown female measures 1.5 to 2 mm. in length and 0.12 mm. in breadth. In the cuticula fine transverse striations are seen. The body has a cylindrical shape of nearly uniform diameter, altho it tapers gradually anteriorly from the part of the esophagus. Posteriorly, the body narrows more abruptly at the region of the anus and becomes a long and fine tip. The tail (from the level of the anus to the end) measures 0.17 to 0.24 mm. in length. The oral orifice is surrounded by four labial palpi, and leads to the oral cavity consisting of a relatively long canal, measuring 0.02 to 0.04 mm. in length. The muscular esophagus has a length of 0.17 to 0.2 mm. It consists of four parts: (1) a relatively long and broad anterior canal; (2) the anterior bulbus; (3) a narrower posterior canal, and (4) the posterior bulbus. The anterior bulbus lies at the middle of the whole length of the esophagus and the oral cavity. The anterior canal region is slightly shorter than the posterior one. The posterior bulbus is 0.02 mm. in diameter and smaller than the anterior. The internal lumen

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of the esophagus gradually narrows posteriorly. The broadest part of it at the anterior end of the anterior canal is 6μ in width, being the same as that of the oral cavity, while its posterior half is apparently closed. It again widens slightly in the anterior bulbus and in the posterior canal region it again closes. The intestine consists of two rows of cells. The two ends of the intestine are thicker than the middle, which occupies the largest part and is compressed by the genital organs. Near the posterior end the intestine narrows abruptly and is connected to the rectum. The excretory canal opens at the level of the posterior bulb of the esophagus.

The worm has a vulva, which opens at about the middle of the body, and paired ovaries and uteri. The ovaries consist of club-shaped rows of cells. The anterior ovary arises slightly anterior to the anus and runs anteriad. Just in front of the middle of the body (or near the posterior end of the esophagus) it narrows and is connected with the uterus. The uterus runs farther anteriad and near the posterior bulb of the esophagus turns posteriad to join the vulva. The posterior ovary arises slightly posterior to the esophagus (almost at the same level as the bend of the anterior uterus) and is continued posteriorly to the uterus, which runs further posteriad. Before it reaches the posterior end of the intestine, it turns anteriad and ends in the vulva. In somewhat younger specimens, the turning point of the posterior uterus and the anus are about 0.16 mm. apart. In the young specimens, each uterus is filled with 10 to 50 eggs, the larger specimens carrying more than the smaller. The posterior uterus is somewhat shorter than the anterior, if not the same length. The uteri of a full grown worm are filled witth hatched embryos. The eggs in the uteri are somewhat irregularly ellipsoidal and measure 44 to 28 by 28 to 32μ.

Full grown males measure 0.9 to 1.2 mm. in length and 0.03 to 0.05 mm. in breadth. They are similar in form to the females. The posterior part narrows abruptly at the region of the bursa copulatrix and forms the tail which curves somewhat laterad. The anus and the posterior end of the body stand 0.064 to 0.07 mm. apart. The tail is 28μ long. The alimentary canal is similar in structure to that of the female, but the esophagus of the male is shorter and smaller than that of the female, measuring 0.13 to 0.14 mm. in length. The male has one testis, which arises directly posterior to the esophagus and runs posteriad, forming the vesicula seminalis. It has two spicules of the same size, 35 to 41μ long. The terminal part of each spicule curves exteriorly and ventrally, and has the shape of a sickle. Dorsally to the spicules, a rod-shaped gubernaculum is present.

A bursa copulatrix is present. The anterior end of this organ lies at about the same level as the anterior end of the spicules and the

posterior end reaches to the place where the body narrows abruptly. It is narrow and is provided with six pairs of stalked papillae; two of them are preanal, while the third pair lies almost at the same level as the anus. The first and the second pairs, and the third and the fourth pairs lie closer together than the second to the third, and the fourth to the fifth pairs. The fifth and the sixth pairs lie closest together of all.

The youngest larvae measure 0.24 to 0.3 mm. long by 0.012 to 0.03 mm. wide. They have an esophagus, which is relatively long, occupying about one third of the entire body length; it contains two bulbi. The oral cavity can be clearly recognized.

The worms were found parasitic in the pupils of the primary schools in the counties of Soma and Inashiki. Seventeen cases out of 668 examined (i. e., 2.5 per cent.) were infected. All the cases were boys and girls between 10 and 14. The examination was carried out during October and November. The worms were found in considerable numbers. Furthermore, Dr. O. Takaki found 3 cases out of 471 pupiles examined in the primary schools of the county of Kitasoma. His examination was made in June, 1914.

It is interesting to note that four pupils out of the seventeen who had been proved to harbor the worms by fecal examination in October and November, 1913, were all found free from the worm on their re-examination in January, 1914. In other words, the worms had entirely passed out of the body without any medical treatment in the course of two to three months. This fact shows that the worm may not be a true parasite, but happened to find a temporary or accidental lodgment in the human body. Still it seems to be highly probable that the worms can thrive in the human alimentary canal, for they were found abundantly and in all the stages of their development. The parasitologic importance of the species is to be studied in the future.

This nematode occurs in the larval and adult forms in the human feces, but no eggs are found in the feces, and therefore it should never be mistaken for *Strongyloides stercoralis* Bavay. Several Japanese articles regarding the discovery of *Strongyloides stercoralis* that were published some time back seem to have been dealing with this worm, especially those of Iwaya, Hasegawa and Chikada, and Shiga (see Japanese references below).

Dr. Takaki reports that these nematodes seem to have no pathogenicity towards their hosts, for the latter endure the presence of innumerable worms without the least sign of ill health.

Fig. 4

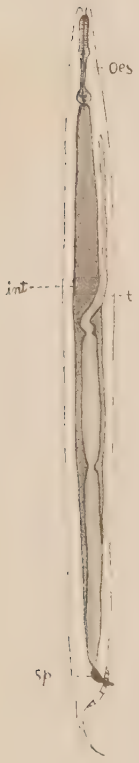


Fig. 1

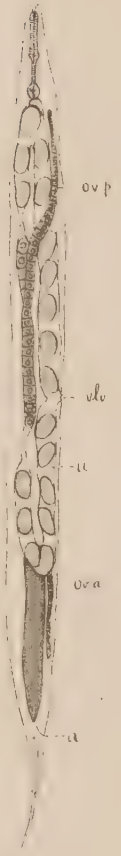


Fig 2

Fig 3

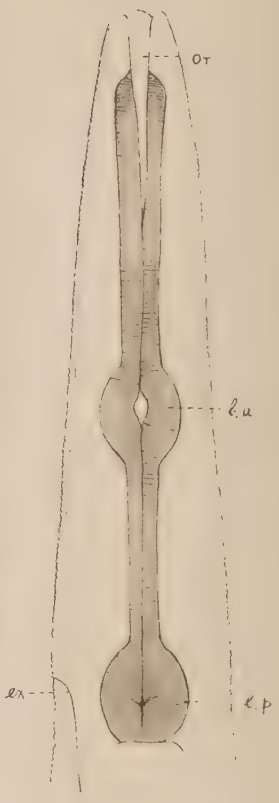


Fig 5

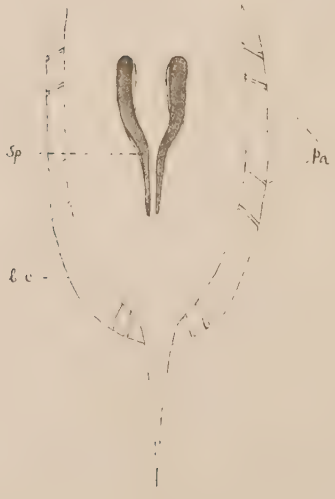
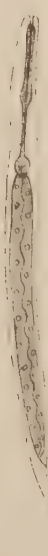


Fig 6



H. Adam . lit. del.

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Explanation of Plate XIII

Fig. 1.—Young female. × ca. 100.

Fig. 2.—Full grown female. × ca. 100.

Fig. 3.—Ditto; the oesophagus. × ca. 50.

Fig. 4.—Full grown male. × ca. 100.

Fig. 5.—Ditto; the posterior end. × ca. 500.

Fig. 6.—Larva. × ca. 300.

Reference Letters

a, annus; *ba*, anterior bulbus; *bp*, posterior bulbus; *bc*, bursa copulatrix; *ex*, terminal part of the excretory duct; *int*, intestine; *oes*, esophagus; *ov a*, anterior ovary; *ov p*, posterior ovary; *or*, oral cavity; *sp*, spicule; *t*, testis; *vlv*, vulva; *u*, uterus.

SPIROCHAETA RECURRENTIS: A FILTER PASSER

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Spirochaetes in an infective form can be forced through a Berkefeld filter by pressures of 50 pounds, and over, to the square inch (Todd and Wolbach, 1914). This note records an endeavor to ascertain the form in which *Spirochaeta recurrentis* passes through the filter.

Wolbach (1915) has shown, by sections, that *Spirochaeta elusa* is present everywhere in the walls of a Berkefeld filter through which these organisms have passed. Nine attempts were made to see *Spirochaeta recurrentis* in Berkefeld filtrates. Since all the experiments were of the same character, the description of one describes all.

(Experiment 595). Two, or more, rats were chloroformed. Heart blood was pipetted off. Organs were ground up with sharp sand in a 1 per cent. solution of sodium citrate in normal saline. The blood, with enough citrate solution to prevent coagulation and the ground-up organs, was passed through a well-impacted Buchner filter in order to remove red cells and organ débris. To prove the presence of spirochaetes in the Buchner filtrate specimens were examined and, to prove the infectivity of the spirochaetes seen, rats were inoculated. The growth of *Bacillus prodigiosus* from a four-day-old culture was then washed into 5 ccm. of normal saline and added to the Buchner filtrate. Control tubes, in which *B. prodigiosus* grew invariably, were inoculated from the filtrate. The Buchner filtrate was then passed through a Berkefeld filter under pressure varying from 50 to 90 pounds. To prove that the filters were intact culture tubes were inoculated with the filtrate; *B. prodigiosus* grew in none. To prove the infectivity of the Berkefeld filtrate rats were inoculated with it. To ascertain the form in which spirochetes existed in the filtrate it was examined.

In seven experiments, rats inoculated with Berkefeld filtrate became infected (Todd and Wolbach, 1914); in only two, of these seven, experiments were spirochaetes seen in the Berkefeld filtrate. In all nine experiments, infective material came from rats which were at the height of a first attack by one of four strains of *Spirochaeta recurrentis*. Each of the strains was known to produce a marked infection in white rats. The filters employed were either "W" or "N" Berkefeld filters. "W" filters were used for both experiments in which spirochaetes were seen in the filtrate.

There are two methods of proving the presence of spirochaetes. The first is the inoculation and demonstrated infection of a susceptible animal. The second is the detection of the parasites by microscopical examination. Both methods are fallacious. Each may reveal an infection where the other fails to do so.

The inoculation of susceptible animals is not an infallible test for the presence of spirochaetes. In these experiments, the strains

employed usually produced heavy infection in white rats, with many spirochaetes in the blood; young white rats were used since they are more susceptible to infection than are older ones. Yet, a few rats, shown to be susceptible by subsequent re-inoculation with the same strain, resisted inoculation by material in which spirochaetes were shown to be present and infective. Spirochaetes were present in resistant rats not at all or in numbers too small to be detected by the microscopical examination of blood. In some instances, spirochaetes have been shown to be present in a resistant rat by aspirating, under chloroform, blood from its heart and by inoculating and infecting with it a fresh rat.

Even repeated microscopical examination may fail to reveal spirochaetes in material known to contain them. As a rule, blood is examined for spirochaetes in thick films, dehemoglobinized and stained by some modification of Romanowsky's method. In order to compare this method with the examination of fresh preparations by dark ground illumination, a series of sixty-nine observations was made in which blood that might easily contain spirochaetes was examined simultaneously by the thick film and dark stage methods. There is little to chose between them. Twenty-six examinations were positive by the dark stage method and twenty-five by the thick film; four times spirochaetes were detected by the dark stage method alone, and thrice spirochaetes were found in thick films when they were unseen by dark stage examination. Thin preparations of blood, covered with ¾-inch square coverslips and ringed with vaseline were used for the dark stage examinations. They were always examined soon after they were made. Ten minutes were spent in the examination of each specimen, whether stained or fresh, before a negative examination was recorded.

Spirochaetes are not thrown down by centrifugalization as are trypanosomes (Dutton and Todd, 1905). Yet, spirochaetes may be seen in films of the precipitate thrown down by centrifugalization of fluids (coxal fluid from ticks, blood) in which previous examination failed to reveal them. Spirochaetes were found in precipitates obtained by centrifugalization at slow speeds, 200 to 500 revolutions per minute, for twenty minutes as well as in fluids centrifugalized at higher speeds, 2,000 to 3,000, for long periods, 90 to 240 minutes. Centrifugalization at low speeds was done in a small centrifuge, distance from the center to the bottom of the tubes being 14 cm. Centrifugalization at high speeds was done in a large centrifuge, the distance from the center to the bottom of the tubes being 24 cm. Ordinary urine centrifuge tubes holding 10 c.cm. were usually employed. It is of advantage to centrifugalize in two stages. Fluid is taken from the bottom of the

tubes first centrifugalized and placed for the second centrifugalization in smaller tubes each about 6 mm. in diameter and 13 cm. in length.

In order to ascertain whether all infective spirochaetes can be brought to the bottom of centrifuge tubes by centrifugalization, infective material was centrifugalized at high speeds, from 1,400 to 3,500 revolutions per minute, for periods ranging from twenty minutes to four hours. The top one-fifth or one-third of the fiuid was drawn off with a syringe, so soon as the centrifuge stopped, and injected into rats. In six out of nine experiments rats so inoculated became infected. In seven of these experiments the precipitate was searched for spirochaetes; they were found in three instances. Twice, the spirochaetes seen in the precipitate from fluids centrifugalized at high speeds were broken.

There was no conspicuous peculiarity in the morphology of the spirochaetes twice found in precipitate from centrifugalized Berkefeld filtrates. There were a few small forms, which would have been seen with difficulty in fresh preparations without dark field illumination; but, on the whole, the parasites were, if anything, rather larger than usual.

SUMMARY

1. *Spirochaeta recurrentis* can be forced in its type form through a "W" Berkefeld filter.

2. Centrifugalization, at the speeds and for the times employed, does not throw down all infective forms of *Spirochaeta recurrentis*.

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VARIATION OF THE OVUM (*SARCOPTES SCABIEI*) UNDER COVERGLASS PRESSURE*

FRED D. WEIDMAN

This contribution is made to indicate the degree of alteration in dimensions of biologic specimens which can occur when they are mounted in fluid under a loose coverslip and the fluid is allowed to evaporate. I have frequently noticed that there is some increase; but had no idea that it amounted to as much, expressed in percentages, as is brought out by this test. Photographs were made of a ripe ovum of *Sarcoptes scabiei* at short time intervals as the fluid (water) evaporated, and all dimensions in the several photographs laid off on a scale with a compass. The findings are indicated in the table below. The photographs were taken at about four minute intervals under the heat of an Edinger apparatus.

	Length	Width
Photograph 1.....	0.190 mm.	0.130 mm.
Photograph 2.....	0.200 mm., 5.3% increase	0.138 mm., 6.2% increase
Photograph 3.....	0.208 mm., 9.5% increase	0.145 mm., 11.5% increase
Photograph 4.....	0.214 mm., 12.6% increase	0.150 mm., 15.4% increase

This means that such a difference in length as 12 per cent. and of width of over 15 per cent. is not to be overlooked when measuring biologic specimens either for comparison with known species or in describing new ones, and that the capillary force which is constantly being exerted more and more on a specimen should be remembered. The factor of pressure will of course vary in importance with different classes of material, depending on the shape, size and consistency of the object.

The dimensions recorded for any specimen should in all cases be as close as possible to those of the specimen as it exists in nature, i. e., as it lies in a stratum of fluid with something to protect it from the pressure of the coverglass. This can be accomplished coarsely by placing a minute drop of wax under the corners of the coverglass and warming over the flame as the coverslip is let down into position, or by ringing with vaselin. Finer adjustment of the thickness of the fluid stratum may be later accomplished under the microscope by hot wires applied to the wax pillars. In his work on protozoa, Doflein recommends such protection against pressure.

* From the Laboratory of Dermatological Research, University of Pennsylvania.

NOTES

In the splendid volumes dedicated to Sir William Osler in honor of his seventieth birthday are some contributions of especial interest to parasitologists. Among these may be noted the papers on malaria control by Bass, on preliminary streptothricosis by Bridge, on spirochaetal jaundice by Gwyn, on leukocytes and protozoa by Goodrich, and on the significance of Rickettsia by Strong.

Professor Frank G. Haughwout, who for the past five years has been Professor of Protozoölogy and Chief of the Department of Parasitology of the College of Medicine and Surgery, and in the Graduate School of Tropical Medicine and Public Health, University of the Philippines, has transferred to the Bureau of Science, Manila. In the latter institution Professor Haughwout will be protozoölogist and will have supervision over the work in parasitology which it is planned to conduct on an extensive scale. Professor Haughwout will retain the position of professorial lecturer in protozoölogy in the University.